

ADRENERGIC, CHOLINERGIC, AND VIPERGIC
NEURO-EFFECTOR CONTROL

With Special Reference to High-Frequency
Burst Excitation Patterns

By

Per-Olof Andersson

Lund 1983

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Abstract This study demonstrated that graded autonomic nerve excitation in intermittent high-frequency bursts, simulating the natural firing patterns established from electrophysiological single fibre recordings, is as capable of evoking smoothly graded effector responses up to maximal ones as conventional low-frequency continuous stimulation. This applied to the adrenergically controlled resistance and capacitance vessels in muscle; and to the parasympathetically controlled vasodilatation and secretion in salivary glands, vasodilatation and motility in the rectum, and vasodilatation in the colon. The only exception was the colonic motor response which proved to be insensitive to burst stimulation. The data indicate that the effective physiological excitation frequency range in peripheral autonomic nerves is much wider (0 to > 40 Hz) than previously believed (0 to < 10 Hz), provided the firing occurs in the common pattern of intermittent bursts. The vasodilator control in all of the investigated parasympathetically innervated tissues was shown to be mediated by a cholinergic as well as a non-cholinergic transmitter mechanism. The results provided strong functional evidence for suggesting VIP, but apparently not substance P, as the mediator of the non-cholinergic vasodilatation in the submaxillary gland, the rectum, and the penis; possibly also in the colon, but not in the parotid gland. The haemodynamics and neurotransmitter mechanisms of pelvic nerve induced penile erection in the dog were elucidated in detail. The data indicated that erection is accomplished by two main circulatory events: A very prompt neural dilatation of the penile 'resistance vessels' causing an about 20-fold increase in blood flow, and a more delayed opening of 'shunt vessels' causing a redistribution of a further increased flow into the cavernous bodies, in turn increasing penile volume by more than 100%. The neural dilatation of the penile 'resistance vessels' seemed to be mainly VIPergic, and the 'shunt' opening at least partly cholinergic, in nature.			
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This summary is based on the following publications:

- I. ANDERSSON, P.O. 1983. Comparative vascular effects of stimulation continuously and in bursts of the sympathetic nerves to cat skeletal muscle. *Acta Physiol Scand* 118:343-348.
- II. ANDERSSON, P.-O., BLOOM, S.R., EDWARDS, A.V. & JÄRHULT, J. 1982. Effects of stimulation of the chorda tympani in bursts on submaxillary responses in the cat. *J Physiol* 322:469-483.
- III. ANDERSSON, P.-O., BLOOM, S.R. & EDWARDS, A.V. 1982. Parotid responses to stimulation of the parasympathetic innervation in bursts in weaned lambs. *J Physiol* 330:163-174.
- IV. ANDERSSON, P.-O., BLOOM, S.R. & JÄRHULT, J. 1983. Colonic motor and vascular responses to pelvic nerve stimulation and their relation to local peptide release in the cat. *J Physiol* 334:293-307.
- V. ANDERSSON, P.-O., BLOOM, S.R., EDWARDS, A.V., JÄRHULT, J. & MELLANDER, S. 1983. Neural vasodilator control in the rectum of the cat and its possible mediation by vasoactive intestinal polypeptide. *J Physiol* 344:49-67.
- VI. ANDERSSON, P.-O., BLOOM, S.R. & MELLANDER, S. 1984. Haemodynamics of pelvic nerve induced penile erection in the dog: Possible mediation by vasoactive intestinal polypeptide. *J Physiol*. In the press.

In the text, these papers are referred to by their Roman numerals.

INTRODUCTION

The studies summarized in this paper provide some new information about the neuro-effector control and transmitter mechanisms of the peripheral autonomic nervous system. The investigations refer to the sympathetic vasoconstrictor control of the resistance and capacitance vessels in skeletal muscle (I), and to the parasympathetic control of vascular and secretory response in the submaxillary and parotid glands (II-III), of vascular and motility responses in the colon and rectum (IV-V), and of the vascular events underlying penile erection (VI).

Much of our knowledge about the peripheral autonomic neuro-effector regulation is based on studies of effector responses to electrical nerve stimulation applied in terms of continuous repetitive impulses evenly distributed over the period of excitation (continuous stimulation). Such investigations have demonstrated that the full range of control of most effectors is exerted by continuous stimulation in a low frequency range, from 0 up to 10-15 Hz (e.g. Folkow 1952, Emmelin 1967, Mellander & Johansson 1968, Hultén 1969).

Aspects on the normal physiological discharge rate in peripheral autonomic nerves were given in a detailed study of the sympathetic neuro-effector organization in anaesthetized cats by Folkow (1952), who made the following main findings: First, the magnitude of the effector response (constriction of resistance vessels) during very strong reflex activation of the sympathetic nervous system was found to be equivalent to that evoked by artificial electrical continuous stimulation of the regional nerves at a rate of 6-8 Hz. Second, upon cessation of short-term electrical stimulation, the 'latency period' before the start of the smooth muscle relaxation, and the duration of the blood flow recovery period, were found to be markedly prolonged at frequencies higher than 8 Hz. Such effects might be considered unphysiological. Third, the vasoconstrictor response to continuous stimulation could not be maintained at frequencies exceeding 15 Hz. These findings, largely confirmed also for other autonomic effectors (see below), have led to the conclusion that the maximal physiological firing rate in peripheral autonomic nerves seldom exceeds 6-8 Hz.

However, recent electrophysiological studies of the discharge pattern in single fibres or in very small bundles of peripheral autonomic nerves have revealed that the spike activity quite commonly occurs in short bursts (1-2 s) at high frequencies (up to 60 Hz) separated by quiescent

periods lasting 5-10 s. Intense reflex stimuli could lead to a prolongation of the bursts with shorter intervals, or to increased discharge rate in the individual burst. Such patterns (cf. schematic illustration in Fig. 1) have been observed in both parasympathetic and sympathetic nerves of anaesthetized and conscious animals as well as in conscious human subjects. This can be exemplified by electrical nerve activity observed in the chorda tympani (Kawamura, Matsuo & Yamamoto 1982); in the vagal nerves to the stomach (Davison & Grundy 1978, Miolan & Roman 1974); in the pelvic nerves to the urinary bladder and large intestine (DeGroat, Booth, Milne & Roppolo 1982); and in the sympathetic nerves to the skin and skeletal muscle vasculature in man (see Wallin 1981).

The question arises whether such autonomic activity patterns are capable of evoking as effective and as strictly graded effector responses as those which have been demonstrated to occur in response to continuous stimulation at graded impulse rates. This problem was approached, as one main objective in the present series of experiments, by analysing various effector responses in sympathetically and parasympathetically innervated organs to electrical high-frequency stimulation in bursts simulating the patterns of discharge recorded electrophysiologically *in vivo* and by comparing such effects with those evoked by conventional low-frequency continuous stimulation. Examples of the applied electrical excitation patterns are illustrated in Fig. 1. Such comparative studies were performed with regard to the sympathetically controlled resistance and capacitance vessels in skeletal muscle (I), and to the parasympathetically controlled vascular and secretory responses in the submaxillary and parotid glands (II, III), and the vascular and motility responses in the colon and rectum (IV, V).

Another problem approached in the present studies deals with possible neurotransmitter mechanisms in the parasympathetic system. It is well known that several parasympathetically controlled effector responses, especially those of vascular and non-vascular smooth muscle, at least in part, are non-cholinergic in nature. Several tentative mediating mechanisms for such effects have been proposed, such as bradykinin formation (e.g. Hilton & Lewis 1955 a, b), or purinergic (Burnstock 1972), serotonergic (e.g. Gershon 1982) and peptidergic (e.g. Bishop et al. 1982), neurotransmission. In the present studies special attention was paid to the possible role of peptidergic mechanisms in parasympathetic vasodilator reactions. Hence, the interest was focused,

in particular, on vasoactive intestinal polypeptide (VIP) and substance P which are considered to exert inhibitory effects on vascular smooth muscle (e.g. Euler & Gaddum 1931, Said & Mutt 1970), and whose presence has been demonstrated morphologically in nerve structures in the vicinity of parasympathetically controlled blood vessels (e.g. Pearse & Polak 1975, Larsson et al. 1976, Bishop et al. 1982).

In the present study an attempt was made to provide functional evidence for the possible role of VIP and substance P as neurotransmitters of parasympathetic vasodilatation in the submaxillary and parotid glands (II-III), in the colon and rectum (IV, V), and in the penis (VI). This was done by analysing the vascular effector responses to close arterial infusions of VIP and substance P, by determining the regional output of these peptides from the tissues during parasympathetic stimulation, and by correlating the peptide release to the nerve induced vascular response.

Emphasis was placed on the haemodynamic effects of autonomic nerve excitation in the present series of experiments. Such effects evoked by continuous nerve stimulation have been described in detail in the previous literature with regard to some of the studied organs, viz. skeletal muscle (e.g. Mellander 1960), the salivary glands (e.g. Emmelin 1967) and the colon (e.g. Hultén 1969). Much less is known about the neural haemodynamic events in the rectum and penis.

In the rectum there are only some previous subjective observations of a vasomotor control of blood flow, exemplified by the classical report of Langley & Andersson (1895) of a marked reddening of the rectal mucosa during pelvic nerve stimulation. To permit direct studies of the parasympathetic control of rectal blood flow, in the present studies, a special vascularly isolated rectal preparation in situ was developed, whose responses could be compared with simultaneous ones evoked in an isolated colonic preparation in situ.

Several hypotheses for the haemodynamics of penile erection have been presented in the literature, largely based on anatomical, angiographic and in vitro studies in various species including man and on flow studies in cadavers (for ref. see Christensen 1954, Klinge & Sjöstrand 1974, Newman & Northup 1981) but also on a few more functional haemodynamic studies in experimental animals in vivo (e.g. Henderson & Roepke 1933, Dorr & Brody 1967, Sjöstrand & Klinge 1979). In these previous studies of penile erection in vivo, neural vasodilatation was estimated from change of arterial inflow pressure at constant flow perfusion, or from change of

venous outflow, and the erectile response was derived from recordings of cavernous tissue pressure, or sometimes followed volumetrically. Only occasionally, however, was more than one of these parameters recorded simultaneously, but this seems to be required for more detailed understanding of the complex haemodynamics of penile erection. In fact there is need of simultaneous quantitative data on both arterial inflow, venous outflow, and penile volume change during erection and such analyses were performed in the present study in the dog (VI) during graded pelvic nerve stimulation.

The present investigations demonstrate that high-frequency stimulation in bursts of the peripheral autonomic nerves can be as effective in evoking maximal effector responses as continuous stimulation, and that smoothly graded effector responses can be obtained by variation of the intra-burst frequency, and/or by shortening the interval between the bursts. This applies to sympathetically controlled resistance and capacitance vessels (Results, section 1), and to parasympathetically controlled vasodilatation and secretion in salivary glands (section 2), and vasodilatation in the colon and rectum (section 3). The studies also provided functional evidence in vivo for suggesting VIP, but apparently not substance P, as a neurotransmitter in the parasympathetic vasodilator control in the submaxillary gland (section 2), the large intestine, especially the rectum (section 3) and the penis (section 4).

The haemodynamics of penile erection in the dog were also elucidated (section 4) indicating that the reaction is accomplished by two main circulatory events: A dilatation of the penile 'resistance vessels' causing a greatly increased blood flow; and an opening of 'shunt vessels' causing a redistribution of flow into the cavernous bodies. The former response seemed to be VIP-ergic, and the latter at least partly cholinergic, in nature.

METHODS

Detailed descriptions of the methods employed in the present series of experiments were given in the original papers (I-VI). Below follows a brief summary of the principal methodological arrangements.

Experimental preparations and recordings

Skeletal muscle preparation

The skeletal muscle preparation was used for a comparative study of the constrictor responses of the resistance and capacitance vessels to regional sympathetic vasoconstrictor fibre stimulation continuously and in bursts. Sympathetic cholinergic vasodilator responses were blocked in all experiments by atropine. The investigation was performed in cats anaesthetised with chloralose and urethane and the preparation consisted of the lower hind leg muscles isolated from the surrounding skin. The reactions of the resistance vessels were derived from continuous recordings of the perfusion pressure and the outflow of blood from the popliteal vein which formed the sole draining vessel from the preparation. Blood flow in all present studies was recorded with a photo-electric drop-recorder operating an ordinate writer. The muscle region, was placed in a temperature-controlled (38°C) plethysmograph to permit recordings of capacitance responses from registrations of changes of tissue volume, separated from concomitant transcapillary fluid movements, according to Mellander (1960). Volume recordings were obtained with the aid of a volume displacement recorder described by Grände, Järhult & Mellander (1974). The sympathetic vasoconstrictor fibres to the muscle region were stimulated via bipolar ring electrodes placed around the ipsilateral sympathetic chain fibres at the level of LIV-LV. The registrations in all present studies were made on an electronic recorder (Grass Polygraph or Devices Recorder).

Salivary gland preparations

The submaxillary and parotid gland preparations were used for comparative studies of the vasodilator and secretory responses to regional parasympathetic stimulation continuously and in bursts.

The submaxillary gland preparation was performed in cats anaesthetized with chloralose alone or in combination with urethane. Changes in salivary gland vascular resistance were derived from perfusion pressure and the venous outflow, the latter recorded from the external jugular vein after ligation of all extraglandular tributaries to this vessel. Salivary secretion was recorded photo-electrically via a cannula inserted in the submaxillary duct. Preganglionic parasympathetic stimulation was performed on the chorda tympani and postganglionic stimulation on the nerve fibres at the hilus of the gland.

The parotid gland preparation was performed in weaned lambs anaesthetized with chloralose. Parotid venous outflow was monitored from the maxillary vein after ligation of all extraglandular tributaries to this vessel and changes of vascular resistance were derived as described in the foregoing. Salivary secretion was recorded via a cannula inserted in the parotid duct. Postganglionic stimulation of the parasympathetic nerve fibres to the gland was performed on the parotid nerve.

Colon and rectum preparations

These preparations were used for a comparative study of the vasodilator and motor responses to parasympathetic pelvic nerve

stimulation continuously and in bursts. The investigations were performed in cats anaesthetized with chloralose and urethane. Some observations were made on an isolated colon preparation, some on an isolated rectum preparation, and some on a combined colon-rectum preparation. In the colon preparation, blood flow was monitored from the colonic vein. In the rectum preparation, blood flow was monitored from a shunt in the inferior mesenteric artery after ligation of all extrarectal branches of this vessel. In the combined colon-rectum preparation, rectal blood flow was recorded as described and colonic blood flow from the superior mesenteric artery after ligation of all extra-colonic branches of this vessel. Vascular resistance was derived from perfusion pressure and the regional blood flows. Motor responses were recorded by means of water-filled balloons inserted in the proximal colon and in the rectum, respectively, in turn connected to a volume displacement recorder (Grände et al. 1974). The parasympathetic nerves to the colon and rectum were stimulated via bipolar ring electrodes placed on both pelvic nerves.

Penile preparation

This preparation was used for a study of the haemodynamics of pelvic nerve induced erection. The investigation was performed in dogs anaesthetized with pentobarbital sodium. The penis was dissected free of skin except for the prepuce and was reflected caudally. Penile venous outflow of blood was recorded from the two dorsal veins of the penis at an outflow pressure of 1-2 mmHg. Erectile responses of the glans penis were recorded volumetrically by placing it inside a transparent cylindrical plethysmograph, sealed proximally by the prepuce which was pulled over the exterior of the cylinder and fixed by a surrounding ligature. By this arrangement the whole glans was kept in fixed position permitting reliable erectile volume change recordings, unaffected by accidental movements or activity of the retractor penis muscle. The plethysmograph was filled with isotonic saline and connected to a volume recording system (Grände et al. 1974). The rate of penile arterial inflow of blood during erection/detumescence was calculated as venous outflow ($\text{ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$) plus/minus the simultaneously recorded rates of erectile volume change ($\text{ml of blood} \times \text{min}^{-1} \times 100\text{g}^{-1}$). The preganglionic parasympathetic nerve fibres to the penis were stimulated via bipolar ring electrodes placed on the two pelvic nerves.

Nerve stimulation characteristics

Efferent stimulation of the severed sympathetic or parasympathetic innervation to the tissues under study was performed with supramaximal square wave impulses delivered from a Grass Stimulator, S 88.

The effector responses were compared during graded conventional low-frequency continuous stimulation and graded intermittent high-frequency burst stimulation, simulating different patterns of electrical spike discharge recorded in vivo (see Introduction and Fig. 1, which exemplifies such patterns). The comparative analyses refer to stimulations continuously and in bursts with the same total number of impulses delivered in unit time, e.g. 4 Hz continuously or 40 Hz in 1 s bursts at 10 s intervals (cf. Fig. 1, A and B). In most cases (Paper I-V) such burst stimulations of 1 s duration at 10 s intervals were employed and graded by varying the intra-burst frequencies. In some studies other patterns of burst stimulation were also used. Thus, in paper I and IV graded burst stimulation was accomplished by varying the interval between bursts (from 2-32 s) at fixed intra-burst frequencies of 16 or 32 Hz (cf. Fig 1 C). In other experiments (IV) graded burst stimulation was

accomplished by varying the burst duration at constant (10 s) burst intervals (see Fig. 1 D)

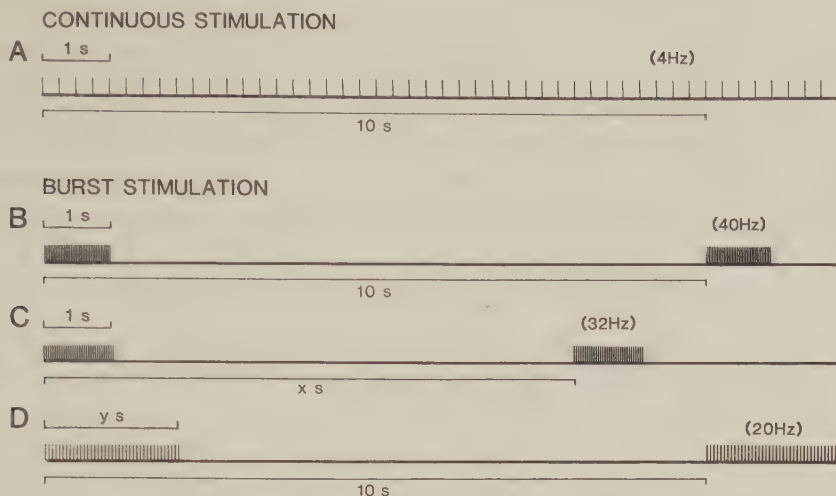


Fig. 1. Schematic illustration of principal stimulation patterns used in the present studies. With all types of stimulation, the same total number of impulses in unit time was delivered.

- A. Continuous stimulation at graded frequencies (exemplified by 4 Hz).
- B. Burst stimulation at graded intra-burst frequencies (exemplified by 40 Hz) at fixed burst duration (1 s) and fixed intervals (10 s).
- C. Burst stimulation at graded burst intervals (x s) at fixed frequency (16 or 32 Hz) and fixed burst duration (1 s).
- D. Burst stimulation at graded burst duration (y s) at fixed frequency and fixed intervals (10 s).

Determinations of nerve induced noradrenaline and peptide outputs

Noradrenaline output from the skeletal muscle preparation was determined during sympathetic vasoconstrictor fibre stimulation. Noradrenaline concentrations in plasma were determined using high pressure liquid chromatography (HPLC) with electrochemical detection (Hallman et al. 1978).

The output of peptides from the salivary glands, colon, rectum, and penis were performed during parasympathetic stimulation and correlated to the concomitant vasodilator responses. For this purpose samples of arterial and regional venous blood were collected in ice-chilled test tubes containing aprotinin (1000 K.I.U. x ml blood⁻¹, Bayer). The samples were immediately centrifuged at +4°C, and the plasma samples stored at -20°C until assayed. VIP, substance P, and somatostatin concentrations were measured by radioimmunoassay (Mitchell & Bloom 1978, Bloom and Long 1982, McGregor & Bloom 1983). The outputs of noradrenaline and the mentioned peptides were calculated as the veno-arterial plasma concentration difference multiplied by the plasma flow.

Statistics

Statistical analyses were based on the Student's t-test or, when comparing three groups of data, on analysis of variance for multiple comparisons according to Scheffe (see Wonnacott & Wonnacott 1977). Additionally, linear regression analysis was used. P-values < 0.05 were considered to be significant. Data are expressed as mean values $\pm$ SEM.

RESULTS and DISCUSSION

The present studies (paper I-VI) provide some new aspects on the peripheral autonomic neuro-effector organization. They are summarized below under four main headings dealing with the sympathetic vascular control in skeletal muscle (section 1), and with the parasympathetic neuro-effector control and neurotransmitter mechanisms in the submaxillary and parotid glands (section 2), the colon and rectum (section 3), and the penis (section 4).

1. COMPARATIVE EFFECTS OF STIMULATION CONTINUOUSLY AND IN INTERMITTENT BURSTS OF THE SYMPATHETIC NERVES TO BLOOD VESSELS OF SKELETAL MUSCLE

The aim of this study was to compare the effects of low-frequency continuous and high-frequency burst stimulation of the sympathetic vasoconstrictor fibres on the resistance and capacitance vessels in cat skeletal muscle. The comparative analysis refers to situations when, with both types of excitation, the total number of impulses in unit time was the same. Various patterns of high-frequency bursts (Fig. 1) were applied, simulating some principal ones observed electrophysiologically *in vivo* (Wallin 1981).

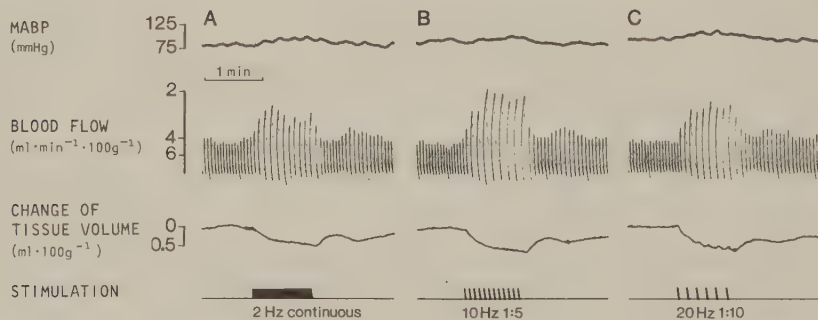


Fig. 2. Original records of mean arterial blood pressure (MABP), blood flow and change of tissue volume from a representative experiment in cat skeletal muscle in response to stimulation of the regional sympathetic vasoconstrictor fibres for 1 min. Panel A shows the effects of stimulation at 2 Hz continuously, panel B at 10 Hz in 1 s bursts at 5 s intervals (10 Hz 1:5) and panel C at 20 Hz in 1 s bursts at 10 s intervals (20 Hz 1:10). Atropine (0.5 mg/kg) was given prior to the experiment.

An example of such an experiment is illustrated in Fig. 2 which shows original recordings of mean arterial blood pressure and blood flow (vascular resistance response) and change of tissue volume (vascular capacitance response) in the muscle preparation in response to

stimulation of the regional sympathetic nerves for 1 min at 2 Hz continuously (A); at 10 Hz delivered in 1 s bursts at 5 s intervals (B); and at 20 Hz delivered in 1 s bursts at 10 s intervals (C). The total number of impulses was thus 120 during the 1 min period of stimulation in all cases. It can be seen that all these three modes of excitation were effective in evoking constrictions of both the resistance vessels and the capacitance vessels and that the magnitudes of these responses were quite similar during these patterns of stimulation.

The burst stimulation was graded in a first series of experiments by varying the intra-burst frequencies, maintaining the intervals between the bursts constant at 5 or 10 s, respectively. The total number of impulses, ranged from 30 to 960/min, corresponding to intra-burst frequencies from 5 to 80 Hz at 5 s intervals and from 5 to 160 Hz at 10 s intervals. For comparison, graded continuous stimulation from 0.5 to 16 Hz was applied, delivering a total number of impulses/min over the same range.

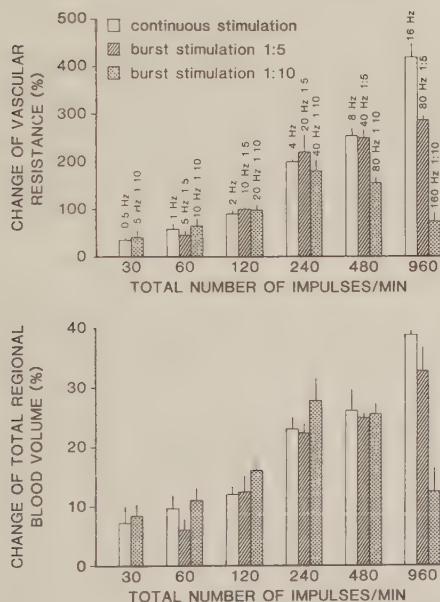


Fig. 3. Comparison of the changes of vascular resistance (resistance response) and total regional blood volume (capacitance response) in cat skeletal muscle in response to stimulation of the regional sympathetic vasoconstrictor fibres for 1 min either continuously, in 1s bursts at 5s intervals (1:5) or in 1s bursts at 10s intervals (1:10) at different frequencies (n=9). Stimulations with the same total number of impulses/min are, for comparison, grouped together.

Fig. 3 shows compiled data on the resistance and capacitance responses to these three modes of graded sympathetic stimulation. Continuous stimulation and burst stimulation at 5 s intervals (1:5) both caused progressive and similarly large increases in vascular resistance with increasing total number of impulses, with the exception that, at the highest frequencies, continuous stimulation (16 Hz) caused a

significantly greater increase in resistance than burst stimulation (80 Hz). Stimulation in bursts at 10 s intervals (1:10) up to 40 Hz also was capable of raising resistance progressively, but at higher rates (80 and 160 Hz) the responses declined and were clearly smaller than those elicited by corresponding continuous stimulations. Intra-burst frequencies of 80 Hz, or more, thus might be considered supraphysiological. Fig. 3, lower panel, shows that continuous and burst stimulation both caused graded responses of the capacitance vessels. Burst stimulation at 160 Hz, however, evoked a clearly submaximal capacitance response.

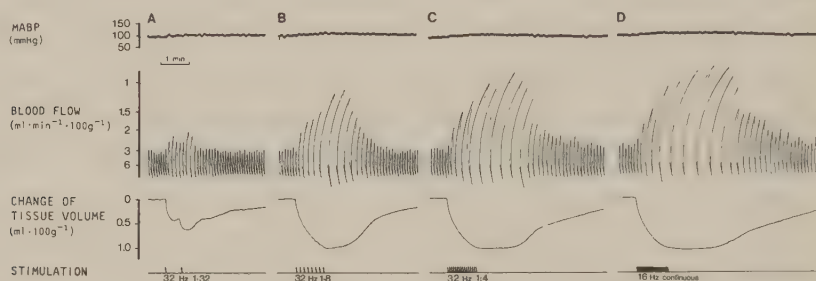


Fig. 4. Original records of mean arterial blood pressure (MABP), blood flow, and change of tissue volume from a representative experiment in cat skeletal muscle in response to stimulation of the regional sympathetic vasoconstrictor fibres for 1 min, either at 32 Hz in 1 s bursts at 32 s intervals (32 Hz 1:32; panel A), at 8 s intervals (32 Hz 1:8; panel B), and at 4 s intervals (32 Hz 1:4; panel C), or continuously at 16 Hz (panel D). Atropine (0.5 mg/kg) was given prior to the experiment.

In a second series of experiments, burst stimulation was graded by varying the interval between the bursts (from 32 down to 2 s), maintaining the intra-burst frequency constant (16 or 32 Hz). Fig. 4 illustrates such an experiment in which the sympathetic nerves were stimulated in 1 s bursts at 32 Hz applied at 3 different intervals, viz. 32, 8, and 4 s. For comparison, continuous stimulation was applied at 16 Hz (panel D). It can be seen that shortening of the interval led to graded augmentation of the vascular resistance and capacitance responses. Burst stimulation at 32 Hz at 4 s intervals caused vascular responses of the same magnitude as continuous stimulation at 16 Hz, despite the fact that in the former case the total number of impulses in unit time was only half of that in the latter. The compiled data from this series of experiments are shown in Fig. 5. It may be noticed that shortening of the interval between the bursts was an effective means of evoking smoothly graded resistance and capacitance responses up to maximal ones.

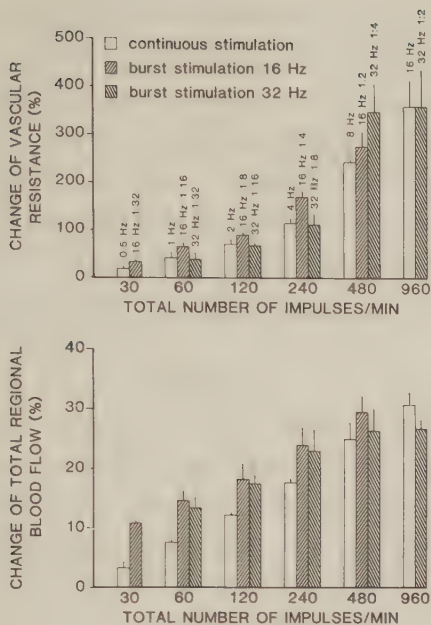


Fig. 5. Comparison of the changes of vascular resistance (resistance response) and total regional blood volume (capacitance response) in cat skeletal muscle in response to stimulation of the regional sympathetic vasoconstrictor fibres for 1 min continuously (0.5-16 Hz), at 16 Hz in 1 s bursts at intervals from 32 to 2 s (16 Hz 1:32-16 Hz 1:2), and at 32 Hz in 1 s bursts at intervals from 32 to 2 s (32 Hz 1:32-32 Hz 1:2), (n=8). Stimulations with the same total number of impulses/min are, for comparison, grouped together.

The output of noradrenaline from skeletal muscle (veno-arterial concentration difference $\times$ plasma flow) in response to stimulation continuously and in bursts was determined in one experiment. Stimulation continuously at 4 Hz and at 40 Hz in 1 s bursts at 10 s intervals evoked similar resistance responses (increment 568 vs. 706%) and similar outputs of noradrenaline (92 vs. 102 $\mu\text{mol} \times \text{min}^{-1} \times 100\text{g tissue}^{-1}$).

Comments

It has been clearly established that electrical stimulation of the sympathetic vasoconstrictor fibres to skeletal muscle, when the impulses are applied continuously, causes maximal vascular effector responses at relatively low frequencies, in the resistance vessels around 15 Hz (Folkow 1952), and in the capacitance vessels at 6-8 Hz (Mellander 1960), a fact confirmed by the present results. Since strong reflex activation of the vasoconstrictor fibres was found to produce vascular effector response no larger than those evoked by continuous stimulation at 6-8 Hz, the conclusion was drawn that the physiological firing rate in peripheral vasoconstrictor fibres in vivo seldom exceeds this range (Folkow 1952).

In view of more recent investigations, however, it cannot be taken for granted that normal sympathetic discharge in vivo occurs as continuous impulses, but rather as intermittent high-frequency bursts. Thus, electrophysiological studies in man, including single fibre

recordings, have shown a normal sympathetic discharge pattern in bursts at 5-8 s intervals with intra-burst frequencies approaching 35 Hz (see Wallin 1981). The present results demonstrated that high-frequency sympathetic burst stimulation mimicking such discharge patterns was capable of evoking graded resistance and capacitance responses up to maximal ones. The magnitudes of these responses were graded both in relation to the frequency of the impulses within each burst (at least up to 40 Hz) and, inversely, in relation to the length of the burst interval (Figs 3, 5).

On the other hand, when such high stimulation frequencies (40 Hz) were applied continuously, the effector responses became submaximal and waned quickly during prolonged stimulation, confirming previous observations by Folkow (1952). The latter author further found that the fading response of the resistance vessels to high frequency continuous stimulation recovered if the stimulation was stopped for only 3-5 s. This finding fits very well with the maintained resistance response observed in this study during repetitive stimulation with high frequency bursts at 2-10 s intervals (e.g. Fig. 3). It appears that such short an interval, achieved by stimulation in bursts, enables the nerve terminals to take up, or synthesize, enough transmitter for repeated release in effective amounts. This opinion may be supported by the present observation that the noradrenaline output in the blood was no less during high-frequency burst, than during low-frequency continuous, stimulation at equal total number of impulses in unit time.

It may be concluded that the vascular smooth muscle effectors in the resistance and capacitance vessels of cat skeletal muscle are responsive to high frequency nerve activation, provided the excitation occurs in intermittent bursts, and that smoothly graded and maintained responses can be obtained by shortening the interval between the bursts or by raising the intra-burst frequency. It is quite likely, therefore, that reinforced constrictions of the resistance and capacitance vessels during natural reflex adjustments may be accomplished by a combination of raised intra-burst frequencies and shortening of the burst interval in the peripheral sympathetic nerves. This does not rule out the possibility that the natural firing in these nerves also can be of the continuous type and graded in the low-frequency range. Yet, the present results provide evidence which indicates that the physiological sympathetic firing range is wider than previously believed, being fully effective at the effector level at least up to 40 Hz in intermittent bursts.

2. COMPARATIVE EFFECTS OF STIMULATION CONTINUOUSLY AND IN INTERMITTENT BURSTS OF THE PARASYMPATHETIC NERVES TO SALIVARY GLANDS

The aim of these studies was to compare the effects of low-frequency continuous and high-frequency burst stimulation of the parasympathetic nerves to the submaxillary and parotid glands with regard to the vasodilator and secretory responses. The secretory responses in both glands are known to be cholinergic in nature. The parasympathetic vasodilatation in the parotid gland is also claimed to be a purely cholinergic response (Kay 1958) whereas that in the submaxillary gland consists of a cholinergic as well as a non-cholinergic component (for ref. see Emmelin 1967). Recent studies indicate that VIP might be the transmitter responsible for the neural submaxillary vasodilatation that persists in the presence of atropine (Bloom & Edwards 1980, Lundberg et al. 1980, Uddman et al. 1980), observations made during continuous stimulation of the chorda tympani. In the present studies, glandular VIP output was determined in response to high-frequency burst stimulation which, among others, provided information about the excitation rates required for release of this neuropeptide. The experiments on the submaxillary gland were made in the cat and the majority of observations refer to preganglionic nerve stimulation, but attempts were also made to excite the postganglionic fibres at the hilus of this gland. A more detailed study of postganglionic nerve effects was made on the parotid gland in which these fibres are more readily excitable, viz. in the parotid nerve. The parotid study was performed in lambs to facilitate adequate recordings of blood flow which is easier to achieve in this species than in cats.

Submaxillary gland (paper II)

Fig. 6 illustrates, in the atropinized cat, comparative effects of preganglionic parasympathetic (chorda tympani) stimulation at either 2 Hz continuously or 20 Hz in 1 s bursts at 10 s intervals on the submaxillary vascular resistance response (upper panel) and the associated VIP output from the gland (lower panel). It can be seen that continuous stimulation caused only a small and waning dilator response, whereas the dilatation to burst stimulation delivering the same total number of impulses in unit time was much more pronounced and maintained throughout the period of stimulation. The mean output of VIP from the gland was substantially

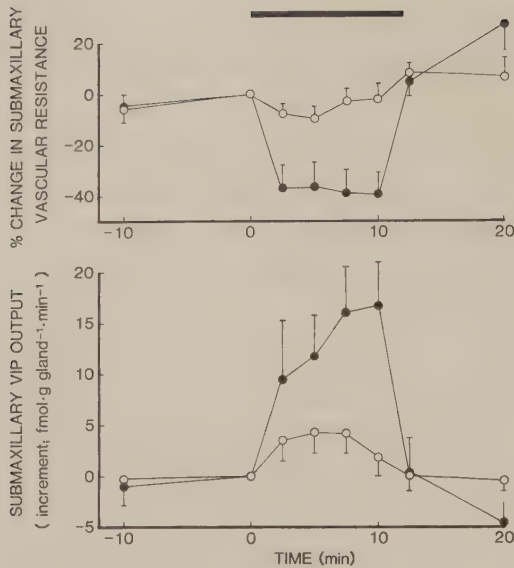


Fig. 6. Comparison of the percentage change in submaxillary vascular resistance (upper panel) and the increase of submaxillary VIP output (lower panel), in response to preganglionic stimulation of the chorda tympani at either 2 Hz continuously for 10 min (open circles; $n=7$), or at 20 Hz in 1 s bursts at 10 s intervals, for the same period (closed circles; $n=8$). Atropine (0.5 mg/kg) was given by i.v. injection 10 min prior to stimulation. Horizontal bar: duration of stimulus.

increased and coordinated in time with the concomitant vasodilator response during stimulation in bursts, whereas the VIP output during continuous stimulation was small and never significantly exceeded the output at rest.

In the absence of atropine, the vasodilator responses to continuous stimulation (2 Hz) and to intermittent burst stimulation (20 Hz), as expected, were larger than the responses after muscarinic blockade shown in Fig. 1, but the VIP output in the absence of atropine, was found to be increased significantly only in response to stimulation in bursts.

The effects in the submaxillary gland of postganglionic parasympathetic stimulation performed on the fibres at the hilus of the gland after ganglionic blockade with hexamethonium were investigated in atropinized cats. Under these conditions, continuous stimulation at 2 Hz proved to be more effective in reducing vascular resistance in the submaxillary gland than did the same pattern of stimulation applied to the preganglionic fibres, a finding that was somewhat unexpected. However, the fall in vascular resistance was consistently greater in response to stimulation in bursts at 20 Hz. Submaxillary VIP output was also consistently greater in response to postganglionic stimulation in bursts than during continuous stimulation at the corresponding lower frequency.

Observations were also made during graded burst stimulation of pre- and postganglionic fibres accomplished by varying the intra-burst frequencies at fixed burst duration (1 s) and fixed interval (10 s). Such observations during preganglionic stimulations made in the absence and

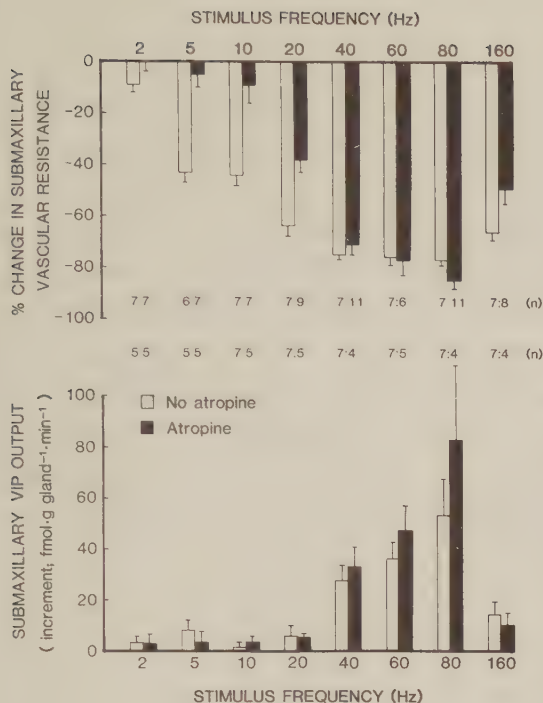


Fig. 7. Comparison of the percentage change in submaxillary vascular resistance (upper panel), and the increase of submaxillary VIP output (lower panel), in response to preganglionic stimulation of the chorda tympani in 1 s bursts at 10 s intervals for 2-3 min, at graded frequencies, in the presence (filled bars) and absence (open bars) of atropine (0.5 mg/kg).

presence of atropine, are summarized in Fig. 7 (upper panel). It can be seen that the vasodilator response was graded in relation to the intra-burst frequency, with maximum obtained at 80 Hz. In the low intra-burst frequency range (< 20 Hz), the vasodilator responses were significantly reduced by muscarinic blockade and, thus, mainly cholinergic in nature. Fig. 7, lower panel, shows the concomitantly observed output of VIP. In the low-frequency range (< 20 Hz), the nerve induced increase in VIP output was very small, but at the higher frequencies (up to 80 Hz) much greater, in the frequency range 20-80 Hz increasing in parallel with the increasing dilator response. At frequencies between 20-160 Hz, the percentage fall in vascular resistance was found to be linearly related to the submaxillary VIP output plotted logarithmically. Atropinization did not seem to affect the neural release of VIP.

The effect of different durations of the individual impulse in the burst was also tested. The submaxillary vasodilator response was found to be unaffected by different impulse durations in the range from 0.1-25 ms.

The submaxillary secretory response to chorda tympani stimulation in 1 s bursts at 10 s intervals in unatropinized cats was found to be graded in relation to the intra-burst stimulus frequency over the range 2-60 Hz. Secretion was entirely abolished by atropine.

Parotid gland (paper III)

In a first series of experiments, the parotid vascular and secretory responses to postganglionic parasympathetic (parotid nerve) stimulation were compared during excitation continuously (1, 2 and 4 Hz) and in 1 s bursts at 10 s intervals at corresponding intra-burst frequencies (10, 20 and 40 Hz) delivering the same total number of impulses in unit time. Stimulation in bursts at either 10 or 20 Hz produced significantly greater flows of parotid saliva, outputs of Na^+ and K^+ , and fall in parotid vascular resistance than continuous stimulation at the corresponding frequencies (1 or 2 Hz). Stimulation at 40 Hz in bursts produced significantly greater output of K^+ and fall in vascular resistance than continuous stimulation at 4 Hz, whereas the salivary flow rates and Na^+ output were closely similar.

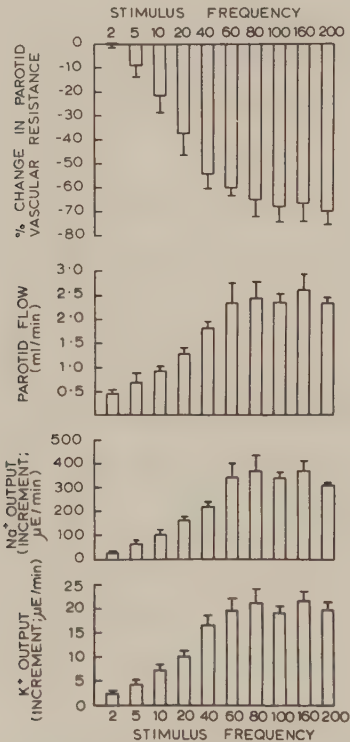


Fig. 8. Relation between parotid vascular resistance, salivary flow, Na^+ and K^+ output and stimulus frequency during stimulation of the parotid nerve in 1 s bursts, at 10 s intervals, for 2-3 min in four weaned lambs.

Fig. 8 shows the parotid responses to postganglionic stimulation in bursts of 1 s duration at 10 s intervals at graded intra-burst frequencies. It can be seen that the vasodilator response and the secretory responses with regard to saliva flow and outputs of Na^+ and K^+ increased with increasing intra-burst frequencies up to maxima at 80 Hz, maintained at further increased frequencies up to 200 Hz.

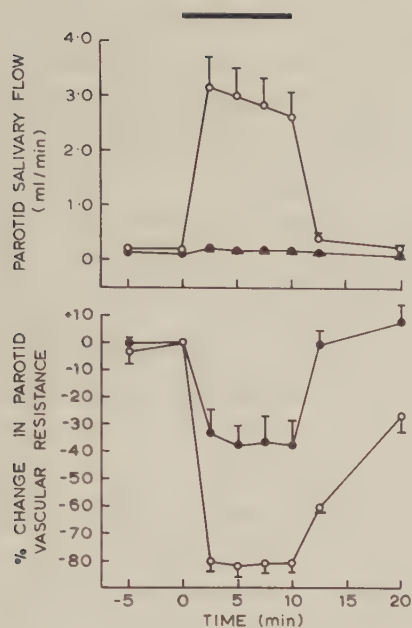


Fig. 9. Comparison of the changes in parotid salivary flow and vascular resistance in eight weaned lambs in response to stimulation of the parotid nerve at 20 Hz continuously for 10 min in the presence (closed circles) and absence (open circles) of atropine (>1.0 mg/kg). Horizontal bar: duration of stimulation.

The contention that the parasympathetic parotid vasodilator response is solely cholinergic in nature (Kay 1958) was re-examined in this study by comparing the effects of continuous stimulation of the parotid nerve at relatively high frequency (20 Hz) before and after effective muscarinic blockade with atropine. Atropine was found to effectively abolish the parotid secretory response to stimulation, confirming the opinion that it is mediated via activation of muscarinic receptors (Fig. 9). However, atropine did not abolish, but merely reduced, the nerve induced fall in parotid vascular resistance, showing that some mechanism other than activation of muscarinic receptors is involved in this response. VIP is a strong candidate for the non-cholinergic vasodilatation in the submaxillary gland, which prompted us to investigate whether it might also be involved in parotid vasodilatation. For this purpose, an analysis was made of the output of VIP from the parotid gland during parotid nerve stimulation; in addition, two other peptides, viz. substance P and somatostatin were analysed. The results were negative, however, insofar that there was no increased output of any of these peptides during parotid nerve stimulation.

Comments

It has been established that maximal secretory and vasodilator responses in salivary glands can be evoked by continuous stimulation of

the parasympathetic nerves at frequencies of 15-20 Hz (Emmelin 1967) and that the highest salivary flow rate that occurs in conscious animals to very strong natural stimuli is matched by electrical continuous stimulation at 6-8 Hz (Emmelin & Holmberg 1967). This has led to the opinion that the physiological firing rates in the parasympathetic nerves to salivary glands only exceptionally exceed 8 Hz.

Recent electrophysiological recordings of the firing pattern in single fibres of the chorda tympani in rabbits (Kawamura et al. 1982) and in postganglionic parotid neurons in sheep (Carr 1977) have shown that the discharge is highly irregular, reaching peak frequencies of about 120 Hz during strong adequate stimulation.

In the present experiments the vascular and secretory effector responses of the salivary glands were studied during stimulation in high-frequency bursts, simulating the type of discharge recorded electrophysiologically in vivo and such effects were compared with those evoked by conventional low-frequency continuous stimulation. The results showed that the vasodilator and secretory responses in the submaxillary and parotid glands were significantly larger during high-frequency burst stimulation than during continuous low-frequency stimulation delivering the same total number of impulses in unit time (Fig. 6). It was further shown that smoothly graded effector responses could be obtained by raising the intra-burst frequency, maxima being obtained at 60-80 Hz (Figs 7, 8). These data demonstrate that the vascular and secretory effectors of salivary glands are gradedly responsive to nerve excitation within a much wider frequency range than previously believed, provided the excitation is applied in intermittent bursts.

Most of the present studies on the submaxillary gland refer to preganglionic stimulation experiments and it might be argued that the demonstrated reinforcement of responses by high-frequency stimulation could be due to facilitation of ganglionic transmission, as reported to occur in the parasympathetic ganglia of the urinary bladder (Booth & DeGroat 1979). However, postganglionic stimulation, as performed most explicitly on the parotid gland, also caused clearly larger responses to high-frequency bursts than to low-frequency continuous stimulation, showing that the reinforcement in salivary glands cannot merely be ascribed to ganglionic facilitation.

In the submaxillary gland the vasodilator response to burst stimulation was found to be atropine sensitive at frequencies between 2 and 20 Hz, and atropine resistant at higher rates (Fig. 7, upper panel).

These findings are in agreement with the observations that the submaxillary vascular response to a single impulse delivered to the chorda tympani is completely blocked by atropine (Emmelin, Garrett & Ohlin 1968) and that the vascular response to continuous stimulation is depressed by atropine in the frequency range up to 5 Hz but unaffected by this drug at 10-20 Hz (Darke & Smaje 1972).

The VIP output from the submaxillary gland was very small in response to low frequency stimulation at rates which caused an atropine sensitive vasodilatation (Fig. 7). At higher frequencies, however, the VIP output became significant and clearly graded in relation to the gradually increasing vasodilator response, a finding which strongly supports the contention that the atropine resistant vasodilatation in this gland is attributable to VIP.

Also in the parotid gland, in which the parasympathetic vasodilatation is previously claimed to be annulled by atropine (Kay 1958), we found an atropine resistant component in the vasodilator response. However, this was not associated with any increase in the output of VIP, substance P, or somatostatin. Thus, the mechanism responsible for the non-cholinergic parotid vasodilatation remains unclear.

3. PARASYMPATHETIC VASODILATOR AND MOTOR CONTROL IN THE COLON AND THE RECTUM

This section summarizes results of studies (IV, V) which indicate that the parasympathetic neuro-effector organization in the two sections of the large intestine, the colon and the rectum, in some respects is markedly different. This was evidenced by a comparative study in cats on vascularly isolated colonic and rectal preparations in situ of the blood flow and motor responses to pelvic nerve stimulations applied both continuously and in intermittent bursts and, further, by analysis of the neurotransmitter mechanisms involved in these reactions.

Blood flow under steady-state 'resting' conditions was found to be about twice as great per unit weight in the rectum as in the colon, indicating a denser vascularization, or possibly higher resting metabolism, in the former segment.

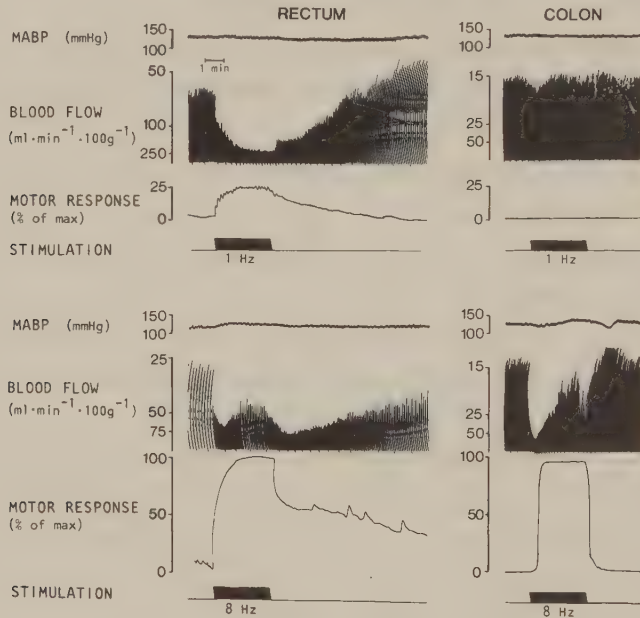


Fig. 10. Effects on mean arterial blood pressure (MABP), blood flow and motor responses in the rectum (left panels) and colon (right panels) of continuous stimulation of the pelvic nerves for 3 min at 1 Hz (upper panels) and 8 Hz (lower panels). Data for rectum and colon taken from different experiments.

Characteristic patterns of the vascular and motor responses to continuous pelvic nerve stimulation are illustrated in Fig. 10. In the rectum, stimulation at 1 Hz (upper left panel) evoked a very marked vasodilatation which was maintained throughout the period of stimulation. Concomitantly, there was a clear-cut contractile motor response. When

stimulation was discontinued the dilator response waned gradually. This pronounced dilator response in the rectum contrasted to the small, transient dilatation seen in the colon to stimulation at 1 Hz (upper right panel). Further, such stimulation was ineffective in evoking any colonic contractile motor response. Continuous stimulation at 8 Hz (lower panels) evoked pronounced vasodilator responses in both the rectum and colon, but they tended to fade during maintained stimulation. This was partly due to mechanical interference with blood flow by the concomitant marked contractile motor responses, but in the colon also due to an 'autoregulatory escape from vasodilator fibre influence' (see below).

The data demonstrate that there is a much more effective pelvic neural control of the resistance vessels in the rectum than colon. Maximal dilator effects in the rectum were evoked by continuous stimulations at 2-4 Hz, corresponding to blood flow values of $200-250 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$ at normal perfusion pressure. In the colon, stimulation at 8-16 Hz was required to obtain maximal dilatation and the peak flow rates then rarely exceeded $150 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$. The frequency response curves for the dilator and motor responses in the rectum were found to be displaced to the left of those in the colon, indicating that the pelvic neural control of the rectum is mainly effected by low-frequency and that in colon by high-frequency, continuous stimulation.

The effects of continuous stimulation (1-16 Hz) were compared with those of high-frequency stimulation (10-160 Hz) in bursts of 1 s duration applied at 10 s intervals. For each comparison the same total number of impulses were delivered in unit time. Original records of the responses to stimulation in bursts (10 and 80 Hz) are shown in Fig. 11. Stimulation in bursts produced quite similar vasodilator response patterns in both the rectum and the colon to those during continuous stimulation at corresponding frequencies (cf. Fig. 10). The rectal contractile motor response during burst stimulation was also quite similar although somewhat attenuated compared to that during continuous stimulation. In the colon, however, stimulation in bursts caused no, or only very small, contractions, maximally comprising 10% of those elicited during continuous stimulation. The transient nature of the concomitant blood flow response in the colon during stimulation in bursts at 80 Hz shows that the fading cannot merely be ascribed to mechanical interference of colonic contraction with the blood flow, but rather to an 'autoregulatory escape from vasodilator fibre influence'.

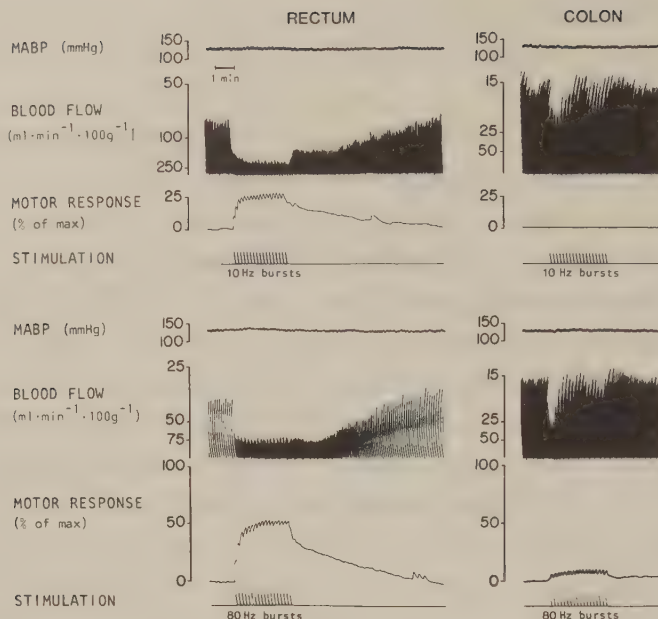


Fig. 11. Effects on mean arterial blood pressure (MABP), blood flow and motor responses in the rectum (left panels) and colon (right panels) of pelvic nerve stimulation in 1 s bursts at 10 s intervals for 3 min at frequencies of 10 Hz (upper panels) and at 80 Hz (lower panels). Observations made on the same animals as in Fig. 10.

The vascular and motor responses to pelvic nerve stimulation were also studied after muscarinic blockade with atropine. Continuous pelvic nerve stimulation under these conditions still caused a substantial vasodilator response in both regions of the large intestine which, with respect to magnitude and time course, approximated to those recorded in the absence of atropine. The only difference was that, following an early peak, the rectal vasodilator response regularly diminished, but it was never abolished during maintained stimulation, as was the case in the colon. The rectal motor response after muscarinic blockade consisted of a small but clearcut initial relaxation followed by a secondary gradual contraction. In the colon, stimulation invariably caused a pure contraction, but this was always delayed and the delay was inversely related to the stimulus frequency. These patterns of motor responses in the rectum and colon basically confirm previous findings by others (e.g. Fasth et al. 1980).

The rectal and colonic blood flow and motor responses to stimulation in bursts after muscarinic blockade were similar to those caused by continuous stimulation at corresponding frequencies with one important exception: In the colon burst stimulation failed to produce any appreciable contractile motor response. This interesting 'uncoupling'

between motor and vascular effects during high frequency burst stimulation of the pelvic nerves in the colon was subjected to a more detailed investigation in paper IV to which the reader is referred.

It may be concluded from the presented results that the vasodilator and motor responses to pelvic nerve stimulation in the colon and rectum are mediated via a cholinergic as well as a non-cholinergic mechanism. The results further indicated that the non-cholinergic responses were not mediated by adrenergic mechanisms or bradykinin formation (IV, V). The non-cholinergic motor responses might be ascribed to several putative neurotransmitters (see Introduction), whereas the mechanisms for the non-cholinergic vasodilatation might be more limited in number, below tested with regard to possible neurotransmission by VIP and substance P. Nerve structures containing these peptides in the vicinity of blood vessels have been demonstrated morphologically (e.g. Buffa et al. 1978, Andersson & Uddman, unpublished).

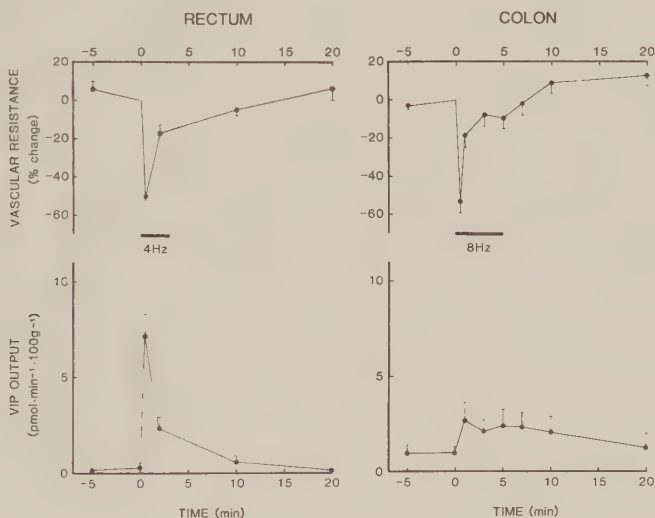


Fig. 12. Changes in vascular resistance and output of VIP from the rectum (right panels; $n=7$) and the colon (left panels; $n=5$) in response to pelvic nerve stimulation in the presence of atropine (1 mg/kg) at 4 Hz and 8 Hz, respectively.

The output of VIP (veno-arterial plasma concentration difference $\times$ plasma flow) from the colon and rectum during optimum pelvic nerve stimulation (8 and 4 Hz, respectively) is illustrated in Fig. 12. It can be seen that in the rectum there was a substantial output of VIP which was correlated in magnitude and duration to the neural vasodilator response. In the colon the pelvic nerve induced VIP output per unit weight was considerably smaller but, on the other hand, sustained for

considerable time after cessation of the stimulation and, hence, poorly correlated in time to the transient neural colonic vasodilatation. In neither segment of the large intestine was the VIP output correlated to the neural motor responses.

There was no significant increase in the output of substance P from the rectum or the colon during pelvic nerve stimulation.

Intra-arterial infusions of VIP, which reproduced the nerve induced increases in venous VIP concentration, caused in the rectum a vasodilator response which closely resembled that during nerve stimulation after cholinergic blockade; in the colon VIP in such doses caused no vasodilator response whatsoever. Close i.a. infusions of VIP in higher concentrations caused dose-dependent vasodilator responses in the rectum which matched the maximal responses to pelvic nerve stimulation. High doses of VIP also were capable of causing colonic vasodilatation but comparative studies indicated that the rectal resistance vessels were some 50- to 100-fold more sensitive to this peptide than the colonic resistance vessels (V). Infusions of VIP, even in very high concentrations ($> 1 \mu\text{M}$), failed to evoke any motor responses in either rectum or the colon. Substance P infusions evoked a transient ($< 1 \text{ min}$) vasodilator response, and a maintained contractile motor response, in both the colon and the rectum. These effects of substance P resembled the contractile motor responses in the colon and rectum, and possibly also the colonic dilator response, evoked by pelvic nerve stimulation after muscarinic blockade. However, substance P failed to mimic the non-cholinergic neural vasodilator response in the rectum.

Comments

The present study, performed in cats, is the first so far as we are aware, which attempts to provide a quantitative analysis of the rectal vascular response to pelvic nerve stimulation and compare that directly with corresponding events in the colon. The results indicate that the vasodilator control by the pelvic nerves is much more effective in the rectum than in the colon and suggest, also in some other respects, a partly different parasympathetic neuro-effector organization in these two regions of the large intestine. Possibly the most distinct difference observed in the present study was that the rectal vasodilatation was maintained throughout the period of pelvic nerve stimulation and, thereafter, returned to the control level quite slowly, whereas the

colonic neural dilator response was very transient. This dissimilarity was observed both with continuous low-frequency and intermittent high-frequency nerve stimulation. These two patterns of excitation were chosen to mimic the discharge in vivo recorded from single preganglionic parasympathetic sacral neurons in this species (DeGroat et al. 1982): Some fibres discharge continuously at low frequency (1-4 Hz), whereas others fire in short intermittent bursts at intra-burst frequencies up to 60 Hz. The present results showed that the vascular smooth muscle effectors in the colon and rectum were gradedly responsive to stimulation in bursts at frequencies up to 60 Hz (Fig. 11), previously considered to be supraphysiological (see Introduction).

Pelvic nerve stimulation, continuously and in bursts, elicited pronounced contractile motor responses in the rectum. In the colon, however, only continuous stimulation caused consistent contractile responses, whereas stimulation in bursts was ineffective. The reason for this 'uncoupling' between vascular and motor responses to burst stimulation in the colon is unclear. Quite likely, the vascular and motor effects might be controlled by different fibre systems, and the 'uncoupling' might be caused by neurotransmission failure in the motor fibres, for instance at ganglionic level. Differences in autonomic effector responsiveness to these two patterns of stimulation have recently been confirmed by Grundy and Scratchard (1982) who adopted the present stimulation technique for a study of the vagal control of gastric motility and acid secretion in the ferret. These authors showed that stimulation in bursts elicited larger muscular contractions but significantly smaller acid secretory responses than continuous stimulation delivering the same total number of impulses in unit time.

The present results demonstrated that pelvic nerve induced vasodilatation in the rectum is mediated by a cholinergic and a non-cholinergic mechanism. A similar dual vascular control has previously been established in the colon (e.g. Hultén 1969). Our results tended to refute the possibility that the non-cholinergic rectal vasodilatation is mediated by catecholamines, bradykinin formation, or substance P, but provided the following evidence for VIP as a neurotransmitter. This peptide, known to be present in nerve fibres in the vicinity of blood vessels in the large intestine (see above), was found to be released from the rectum in response to stimulation of the pelvic nerves in amounts and with time characteristics well correlated to the concomitant vasodilator response. Moreover, i.a. infusion of VIP in amounts which mimicked the

peptide concentration in the rectal venous effluent during pelvic nerve stimulation, evoked vasodilator response of similar magnitudes and time characteristics to those elicited by stimulation. Further, the rectal vessels were found to be some 50-100 times more sensitive to infused VIP than the colonic vessels.

VIP might possibly also be involved in colonic vasodilatation but the evidence was less convincing. Thus, the neural VIP release from the colon per unit weight was substantially less than from the rectum and it was poorly correlated to the colonic vasodilator response (Fig. 12).

VIP has been suggested to mediate the atropine resistant contractile motor response to pelvic nerve stimulation in the colon (Eklund et al. 1979) but our data failed to support this hypothesis since infusion of this peptide, even in large doses ($> 1 \mu\text{M}$), caused no discernible motor effect in the colon, nor in the rectum. Substance P infusions, on the other hand, evoked contractile motor responses in both the colon (cf. Hellström & Rosell 1981) and the rectum which resembled the atropine resistant responses to pelvic nerve stimulation. Although no significant output of substance P was obtained during stimulation, this peptide is known to be rapidly inactivated in vivo (e.g. Pernow 1955) and a local release of substance P is therefore not necessarily discernible in the venous effluent blood. Thus, the possibility remains that substance P might be involved in the contractile motor responses of the large intestine.

4. HAEMODYNAMICS OF PELVIC NERVE INDUCED PENILE ERECTION IN THE DOG: POSSIBLE MEDIATION BY VIP

The haemodynamics of penile erection and the neurotransmitter mechanisms of the underlying circulatory events are not fully understood. The aim of this study was to elucidate, in the anaesthetized dog, the haemodynamics of pelvic nerve induced penile erection by analysing in quantitative terms the changes of penile arterial inflow, venous outflow and tissue volume during graded nerve excitation. The study also provides information on possible neurotransmitter mechanisms involved in the erectile response.

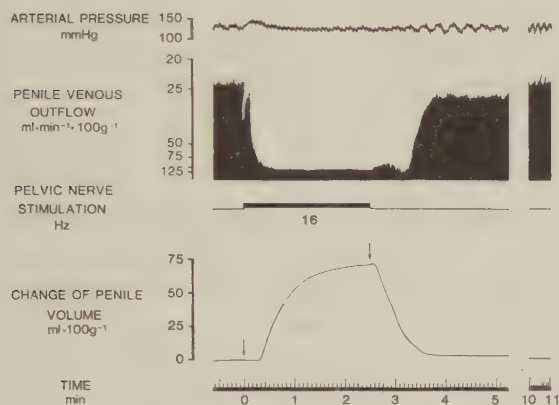


Fig. 13. Effects on arterial pressure, penile venous outflow, and penile volume during erection in the dog evoked by optimum stimulation of the pelvic nerves (16 Hz). Arrows denote start and end of stimulation. Note the prompt onset of the penile blood flow increase and the delayed start of the erectile volume increase.

The penile haemodynamic responses to pelvic nerve stimulation at optimum frequency (16 Hz) are illustrated in Fig. 13. Nerve stimulation caused an almost instantaneous and very marked penile vasodilator response leading to an increase in venous outflow to a value of $110 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$ in 40 s, maintained throughout the stimulation period. Penile volume was unaffected during the very early phase of this vasodilator response, but after a delay of 20 s nerve stimulation caused an abrupt erectile response. The rate of penile volume increase was very fast initially ($90 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$) and then more gradual. This tumescence of the penis (volume increase 70%) was apparent also in marked increases in penile length, circumference and turgidity, and appeared to represent a maximal erectile response under these experimental conditions. Cessation of nerve stimulation led to a rapid disappearance of the erectile response, penile volume approaching the pre-stimulatory value in about 1 min.

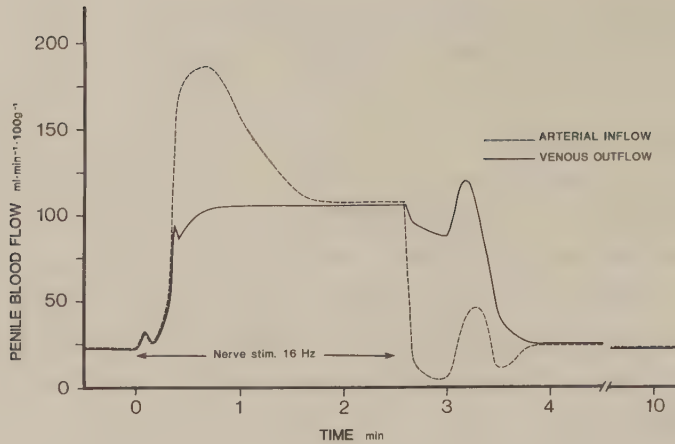


Fig. 14. Changes of penile arterial inflow and penile venous outflow during erection in the dog evoked by optimum stimulation of the pelvic nerves (16 Hz). Data taken from the experiment depicted in Fig. 13. Arterial inflow calculated from venous outflow values and concomitantly observed rates of penile volume change. Arterial inflow exceeds venous outflow during the phase of erectile cavernous filling and is lower than venous outflow during the phase of penile detumescence.

Fig. 14 shows in greater detail the circulatory events underlying erection. It depicts a replotted version of the original venous outflow curve in Fig. 13 and, in addition, the concomitant change in penile arterial inflow. The latter was derived quantitatively from the recorded venous outflow and the rates of penile volume change during erection/detumescence, in turn caused by pooling/emptying of blood in the cavernous bodies. It can be seen that arterial inflow was identical to venous outflow until the onset of penile erection. During the phase of cavernous filling, however, arterial inflow greatly exceeded venous outflow, during the transient peak almost by a factor of 2. After this peak, arterial inflow declined again and approached venous outflow during the steady state of full erection. Cessation of pelvic nerve stimulation led to quick penile detumescence and, concomitantly, to a very abrupt and marked decrease in arterial inflow followed by a transient oscillatory flow pattern before flow stabilized at the pre-stimulatory control level. The decrease in venous outflow was more delayed during the period of detumescence due to the addition of blood from the emptying cavernous tissue, after which it quickly returned to control.

These results provide evidence for the following two main haemodynamic events in erection: First, there was a prompt dilatation of the penile 'resistance vessels' causing a greatly increased arterial inflow which, however, in the very early (~ 20 s) phase caused no sign of erection and, hence, bypassed the cavernous bodies, thus increasing venous outflow to

the same extent. One consequence of this 'resistance vessel' dilatation must be that arterial microvascular pressure is greatly increased (see below). The second event was the erectile response proper which started with a delay of about 20 s. Apparently it was caused by a sudden opening of low resistant 'shunt vessels' diverting part of the further increased arterial inflow into the cavernous bodies at established high pressure head from the microvessels, thereby causing rapid filling. A complete redistribution of blood flow from the extra-cavernousal vascular system to the cavernous bodies by some constrictor mechanism need not be invoked. If this had occurred it should have been observed as a drastic decrease in venous outflow during the phase of cavernous filling. In fact, there was only a minor transient drop in venous outflow during this period (Fig. 14) which most likely is explained merely by passive elastic vascular recoil at the time of 'shunt' opening.

The effects of frequency-graded pelvic nerve stimulations on penile venous blood flow and penile volume are illustrated in Fig. 15. Excitation at low rates (< 1 Hz) caused no discernible vasodilatation or erectile volume increase whatsoever. Stimulation at 2 Hz evoked a clearcut vasodilator response, but still no erectile response. At 4 Hz there was a more marked increase in venous blood flow and this was now associated with a clearcut, though submaximal, erectile response. Both the vasodilator and erectile responses were larger at 8 Hz and they became maximal at 16 Hz, both responses being well maintained during the periods of stimulation. Excitation at 20 Hz still caused an almost maximal vasodilator response, but the erectile response was only about half as large as that at 16 Hz and the volume increase was not well maintained during the period of stimulation. This impairment of the erectile response at such high rate of excitation was not due to a 'fatigue' phenomenon of the preceding repetitive stimulations, since maximal effects were regained at a stimulation at 16 Hz performed later on in this experiment.

It is concluded that an optimum erectile response in the dog is evoked at a continuous pelvic nerve excitation rate of about 16 Hz; the erection was smaller both at higher and lower frequencies, the very low ones being ineffective. For the whole material, pelvic nerve stimulation at optimum frequency (16 Hz) caused, on the average, a 17-fold increase in venous outflow, a 25-fold increase in peak arterial inflow and a 107% increase in penile volume (VI).

An attempt was made in this study to define the neurotransmitter

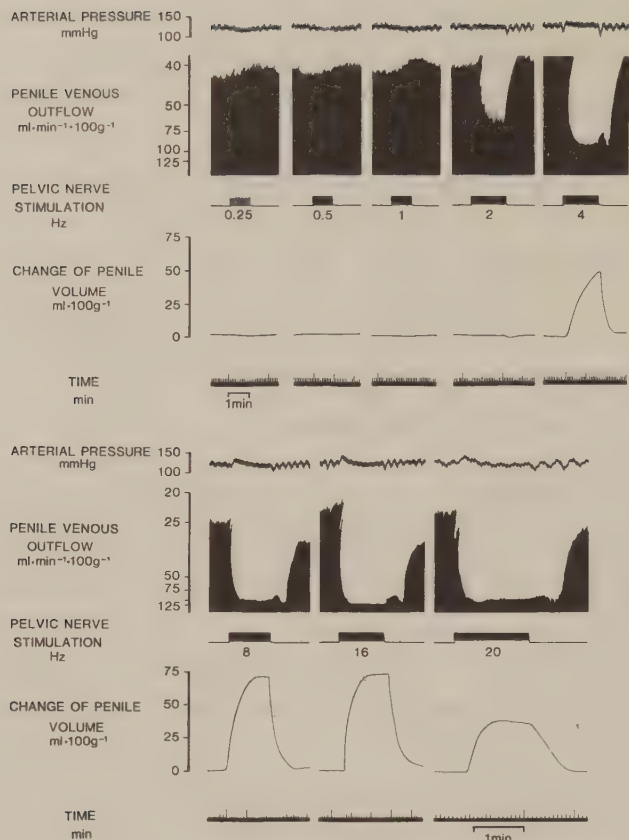


Fig. 15. Effects on arterial pressure, penile venous outflow and penile volume in the dog evoked by graded pelvic nerve stimulations (0.25-20 Hz). The threshold frequency for the blood flow response was 2 Hz and for the erectile volume increase 4 Hz. Both responses became maximal at 16 Hz and declined at 20 Hz. (Note increased paper speed at 20 Hz stimulation).

mechanisms responsible for the described vascular events of erection. This problem was first approached by comparing the pelvic nerve induced penile vascular responses before and after effective muscarinic blockade with atropine (1 mg/kg bwt i.v.). Such experiments demonstrated that, in the atropinized dog, optimum nerve stimulation (16 Hz) still caused a maximal, or nearly maximal, vasodilator response in the 'resistance vessels', whereas the erectile response proper was much curtailed. On the average, atropine reduced the neural erectile penile volume increase from 107 to 41% at optimum excitation, a difference which was statistically significant ($p < 0.05$). Analysis of the penile frequency-response curves in the absence and presence of muscarinic blockade demonstrated that atropinization did not significantly affect the flow ('resistance vessel') responses but markedly depressed the erectile responses over the entire effective frequency range of nerve excitation.

It was concluded that the main pelvic nervous mechanism for dilatation of the penile 'resistance vessels' is largely non-cholinergic in nature, whereas the erectile response related to 'shunt' opening, at least partly, is dependent upon a cholinergic mechanism. This was corroborated by effects observed during close arterial infusion of acetylcholine (VI) which evoked a clearcut erectile response with time characteristics similar to those during pelvic nerve stimulation, and a 'resistance vessel' dilatation which, however, was more sluggish than the neural response.

Previous morphological demonstrations of nerve structures containing VIP (e.g. Larsson, Fahrenkrug & Schaffalitzky de Muckadell 1977) and substance P (e.g. Alm et al. 1978) in the vicinity of vascular smooth muscle in the penis hint at the possible involvement of these peptides in penile erection. In the present study an attempt was made to provide functional evidence in vivo for peptidergic neurotransmission of the vascular events of erection. This was done by analysing the penile vascular effector responses to close arterial infusions of VIP and substance P, by determining the output of these peptides from the penis during pelvic nerve induced erection, and by correlating the peptide release to the erectile vascular events.

Close arterial infusions of VIP or substance P in doses of $1 \mu\text{g}/\text{min}$ produced pronounced increases in penile venous outflow of blood and clearcut, though submaximal, erectile responses. The pelvic nerve induced release of these two peptides during erection was, however, found to be different. Data for the output of VIP from the penis during pelvic nerve stimulation at 20 Hz are illustrated in Fig. 16. It can be seen that stimulation caused a rapid and substantial output of VIP, coordinated in time with the penile vasodilator response, and a rapid decrease in the output upon cessation of the stimulation. At the peak, the VIP output comprised $1370 \pm 390 \text{ fmol} \times \text{min}^{-1} \times 100\text{g}^{-1}$ ($n=8$). These data provide functional evidence in vivo for the hypothesis that VIP is a neurotransmitter involved in erection, perhaps especially in the dilatation of the penile 'resistance vessels'. A corresponding analysis with regard to substance P showed no consistent output of this peptide during pelvic nerve stimulation (VI); such poor correlation to the neural vasodilator response questions a role of this peptide in pelvic nerve induced penile erection.

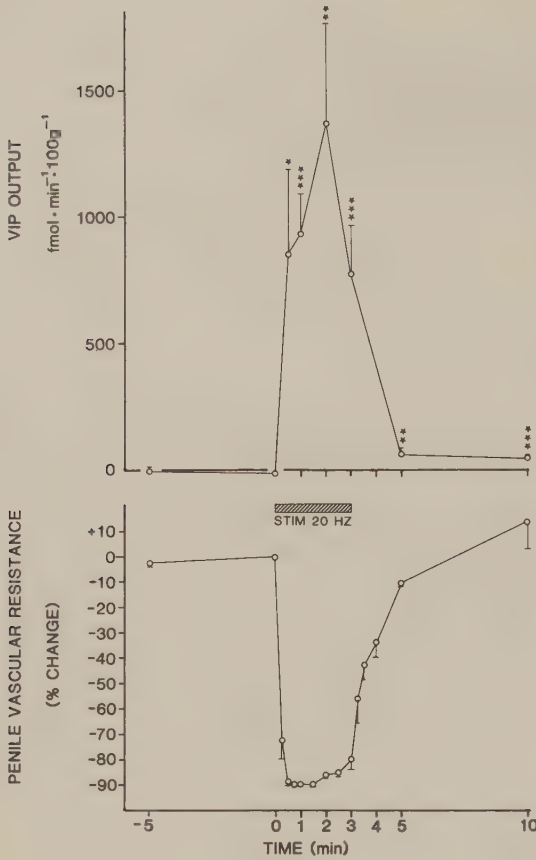


Fig. 16. Output of VIP from the canine penis and change of penile vascular resistance in response to stimulation of the pelvic nerves (20 Hz) in the absence of atropine. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Comments

This study describes in quantitative terms the changes of arterial inflow, venous outflow, and tissue volume in the penis and their time characteristics during pelvic nerve induced erection in the dog. Such combined information about penile circulatory events, not available in the previous literature, may aid in the understanding of the haemodynamics of erection.

The study indicated that pelvic nerve induced erection is accomplished by two main circulatory events: First, there was a prompt dilatation of penile 'resistance vessels' causing greatly increased arterial inflow which in the early phase bypassed the cavernous bodies. Second, the erectile response proper began after a distinct delay of about 20 s. Apparently it was caused by sudden opening of low resistant 'shunt vessels' diverting part of the further increased arterial inflow into the cavernous bodies leading to rapid filling. During the cavernous filling

phase, arterial inflow greatly exceeded venous outflow and returned to the venous outflow level again in the steady state of full erection.

It is quite likely that the dynamics of the cavernous filling/emptying during penile erection/detumescence are influenced by an additional factor, viz. the contractile state of the smooth muscles of the cavernous trabeculae (Sjöstrand & Klinge 1979). Relaxation of these structures would facilitate blood accumulation in the cavernous bodies during erection, and contraction would help to increase the rapidity of detumescence. Analysis of the rates of penile tumescence and detumescence in the present study supported this contention (VI).

The functional significance of the early neural dilatation of the penile 'resistance vessels' seemed to be to establish a high pressure head from the arterial microvessels to the cavernous space, thereby ensuring rapid erection. The 'shunt vessels' to the cavernous bodies, considered to be the penile helicine arteries (Newman & Northup 1981), are estimated from microsphere studies to have a lumen diameter of about 100 μm , when open (Newman, Northup & Devlin 1964). The pressure in microvessels of this size can be calculated to rise well above 100 mmHg at maximal resistance vessel dilatation (cf. Fronek & Zweifach 1975, Grände, Lundvall & Mellander 1977). Such a pressure per se in the helicine arteries would suffice to produce a cavernous tissue pressure of roughly the same magnitude during full erection, which is compatible with the directly observed penile tissue pressure in this state of about 100 mmHg reported by Henderson and Roepke (1933). Blood flow at high intra-cavernous pressure is thus evidently flowing through the cavernous bodies to the venous side during full erection, and a venoconstrictor mechanism is not required for cavernous filling as evidenced by Dorr and Brody (1967). An apparent prerequisite for rapid erection, as noticed in copulative behaviour, is that driving pressure from the penile arterial microcirculation is high at the time of cavernous 'shunt' opening, and such an effect thus seems to be ensured by the described preceding 'resistance vessel' dilatation.

Analysis of the frequency-response characteristics of penile vasodilatation and erection during pelvic nerve stimulation in the dog showed that the threshold frequency for the former was 1-2 Hz and for the latter 2-4 Hz and that maximal effects for both were reached at 16 Hz (Fig. 15). A maximum erectile response was reported to be obtained at much lower rates of pelvic nerve excitation (2 Hz) in the rabbit (Sjöstrand & Klinge 1979) which indicates a marked species difference.

Such a discrepancy might be related to different copulation behaviour in the dog and the rabbit. In the rabbit, this act is very short-lasting with sexual arousal and copulation often completed within less than 20 s (Sjöstrand & Klinge 1979).

The fact that the pelvic nerve induced penile blood flow response and the erectile response in the dog differed both with regard to time of onset and to threshold frequency indicated that the underlying vascular events were governed by partly different regulatory mechanisms. Attempts were made to define the neurotransmitter mechanisms involved. It was demonstrated that muscarinic blockade caused no significant alteration of the pelvic nerve induced flow response but clearly curtailed erectile response over the entire effective frequency range. These data, taken together with observed effects of acetylcholine, indicated that the dilatation of the penile 'resistance vessels' is mainly non-cholinergic in nature, whereas a cholinergic mechanism seems to contribute to the erectile response proper by an inhibitory influence on the penile 'shunt vessels' and/or on the trabeculae of the cavernous bodies. The conclusion that the pelvic neural dilatation of the penile 'resistance vessels' is non-cholinergic in nature confirms previous studies (Henderson & Roepke 1933, Dorr & Brody 1967, Sjöstrand & Klinge 1979). The present study demonstrated that pelvic nerve stimulation caused a substantial release of VIP from the penis which was correlated in onset and duration to the neural vasodilator response (Fig. 16). I.a. infusion of VIP elicited moderate erection, and a penile vasodilator response which resembled the neural response. These in vivo data, viewed in the light of previous morphological observations (Larsson et al. 1977), and in vitro studies on retractor penis and cavernous smooth muscle (Sjöstrand, Klinge & Himberg 1981, Willis et al. 1981), indicate that VIP might be the neurotransmitter responsible for the non-cholinergic pelvic nervous penile vasodilatation. Analogous functional evidence for VIP as a neurotransmitter in the pelvic nerves was recently obtained in the cat, both with regard to neural vasodilatation in the penis (Mellander, Andersson & Bloom 1984) and in an adjacent tissue, the rectum as discussed in Results, section 3. The present results provided poor evidence, however, for suggesting substance P as a neurotransmitter involved in pelvic nervous penile vasodilatation.

It might be emphasized, finally, that erection under physiological conditions probably is associated with a complex activity pattern in several autonomic nerves. This problem was elucidated in a detailed study

in the rabbit by Sjöstrand and Klinge (1979). These authors found that, in this species, there are two vasodilator paths, both with pelvic ganglionic relays, viz. the parasympathetic pelvic nerves and the sympathetic hypogastric nerves which seem to work synergistically to cause erection. The vasoconstrictor nerves from the sympathetic chains were found to exert a strong antagonistic action on the penile dilator effects evoked by the pelvic or hypogastric nerves. The present study was performed in the dog and one should be aware of possible species differences in the neural control, and in the haemodynamics, of penile erection.

SUMMARY

The present investigations provide some new information about the neuro-effector control and transmitter mechanisms of the peripheral autonomic nervous system. This refers to the sympathetic control of resistance and capacitance vessels in skeletal muscle; further, to the parasympathetic control of the vascular and secretory responses in the submaxillary and parotid glands, the vascular and motor responses in the colon and rectum, and the vascular events underlying penile erection.

One main objective was to describe the effector responses to graded autonomic nerve stimulation applied as intermittent high-frequency impulse bursts, simulating single autonomic fibre discharge patterns recently recorded electrophysiologically in vivo. Such effects were compared with those evoked by conventional low-frequency nerve stimulation. The natural firing rates in the intermittent bursts much exceed the limit of 6-8 Hz previously believed to be maximal in the peripheral autonomic nervous system.

In these studies an examination was also made of the role of peptidergic neurotransmitter mechanisms in the non-cholinergic dilator control of the blood vessels in the parasympathetically innervated organs, with special reference to VIP and substance P. This problem was approached functionally by analysing the vascular effector responses to close arterial infusions of VIP and substance P, by determining the regional output of these peptides from the tissues during parasympathetic stimulation, and by correlating the peptide release to the nerve induced vascular responses.

The following main conclusions were drawn:

The comparative analysis of the effector responses to conventional low-frequency (0-16 Hz) continuous stimulation and high-frequency (0-160 Hz) stimulation in 1 s bursts at 10 s intervals (delivering, for each comparison, the same total number of impulses in unit time) showed that the latter mode of excitation can be as effective in evoking maximal effector responses as continuous stimulation. This applied to the sympathetically controlled resistance and capacitance vessels in skeletal muscle, and to the parasympathetically controlled vasodilatation and secretion in the salivary glands, the vasodilatation and motility in the rectum, and the vasodilatation in the colon. The only exception was the

colonic motor response which proved to be insensitive to burst stimulation.

Smoothly graded effector responses were obtained by increasing the intra-burst frequency (maxima at 40-60 Hz) at constant burst intervals, and/or by shortening the interval between the bursts (maxima at 2-4 s) at fixed intra-burst frequency. These data, taken together, indicate that the physiological, effective excitation frequency range in peripheral autonomic nerves is much wider than previously believed, provided the excitation occurs in intermittent bursts.

The vasodilator control in all of the investigated parasympathetically innervated tissues was shown to be mediated by a cholinergic as well as a non-cholinergic transmitter mechanism. The results provided functional evidence for suggesting VIP, but apparently not substance P, as a mediator of the non-cholinergic vasodilatation in the submaxillary gland, the rectum, and the penis; possibly also in the colon, but not in the parotid gland.

The parasympathetic neuro-effector organization in the colonic and rectal segments of the large intestine was found to differ in several respects; an especially marked dissimilarity was the pronounced and well maintained neural vasodilatation in the rectum contrasting to a small and fading response in the colon.

The haemodynamics of pelvic nerve induced penile erection were elucidated in the dog by combined quantitative analyses of arterial inflow, venous outflow, and change of penile volume. The results indicated that there are two main circulatory events underlying penile erection: First, a dilatation of the penile 'resistance vessels' causing a greatly increased blood flow and, second, after a distinct delay of about 20 s, an opening of 'shunt vessels' causing a redistribution of flow into the cavernous bodies. On the average, erection was associated with a 25-fold increase in peak arterial inflow, a 17-fold increase in venous outflow, and a more than 100% increase in penile volume. The 'resistance vessel' dilatation seemed to be mainly VIP-ergic, and the 'shunt vessel' opening at least partly cholinergic, in nature.

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NEURAL VASODILATOR CONTROL IN THE RECTUM OF THE CAT AND ITS POSSIBLE MEDIATION BY VASOACTIVE INTESTINAL POLYPEPTIDE

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SUMMARY

1. Vascular and motor responses in the rectum to pelvic nerve stimulation are described in the anaesthetized cat and compared with corresponding effects observed in the colon. The response comprises a cholinergic and a non-cholinergic component, and an attempt has been made to elucidate the latter.

2. Pelvic nerve stimulation evoked a pronounced and well maintained vasodilator response in the rectum whereas that in the colon was transient. Maximal vasodilation occurred at much lower stimulus frequencies in the rectum (2–4 Hz) than it did in the colon (8–16 Hz) and maximal blood flow under these conditions was also greater in the rectum ($> 250 \text{ ml } 100 \text{ g}^{-1} \text{ min}^{-1}$) than the colon ($< 150 \text{ ml } 100 \text{ g}^{-1} \text{ min}^{-1}$). Muscarinic blockade further curtailed the colonic vasodilator response to pelvic nerve stimulation, whereas the rectal dilatation was only slightly reduced in the presence of atropine.

3. Pelvic nerve stimulation caused a substantial release of vasoactive intestinal polypeptide (VIP) from the rectum, which was related both in magnitude and duration to the vasodilatation. Intra-arterial infusions of VIP, which reproduced this rise in rectal venous VIP concentration, caused a rectal vasodilator response which closely resembled that during pelvic nerve stimulation after cholinergic blockade. The rectal vasculature was estimated to be 50–100 times more sensitive to VIP than the colonic vasculature. VIP therefore seems to be the most likely putative neurotransmitter responsible for non-cholinergic rectal vasodilatation.

4. Stimulation of the pelvic nerves also caused rapid contractile motor responses before, and more gradual motor responses after, muscarinic blockade in both the colon and rectum, in the latter preceded by a non-cholinergic relaxation. These patterns of motor activity largely confirm previous results. Infusions of substance P effectively mimicked the non-cholinergic contractile motor responses but we failed to demonstrate significant release of this peptide during pelvic nerve stimulation in the present

The experimental work was performed at the Department of Physiology and Biophysics, University of Lund, Sweden.

experiments. However, substance P is rapidly inactivated and might possibly be involved in these responses.

5. Stimulation of the pelvic nerves in bursts at high frequencies (up to 80 Hz), simulating a discharge pattern observed electrophysiologically *in vivo*, was effective in eliciting all the above responses, with the exception of the colonic contraction.

INTRODUCTION

The autonomic nervous control of blood flow in the gastrointestinal tract has been studied in great detail in most regions other than the rectum which is less accessible for the purpose of direct blood flow recordings. However, there are some early indirect observations suggestive of a highly effective vasomotor control of rectal blood flow, exemplified by the classical report of Langley & Anderson in 1895 of a marked reddening of the rectal mucosa during pelvic nerve stimulation.

In the present study the rectal vasodilator response to pelvic nerve stimulation was studied directly in anaesthetized cats and an attempt was made to define the mediating mechanisms. The neurogenic vascular and motor responses in the rectum were compared with those in the colon. The peripheral ends of the pelvic nerves were stimulated for 2–5 min either continuously at low frequencies (1–16 Hz) or in intermittent bursts at high frequencies (10–160 Hz), simulating two principal types of discharge pattern observed electrophysiologically in single pelvic nerve fibres (de Groat, Booth, Milne & Roppolo, 1982). The results show that the parasympathetic vasodilator response is both greater and more prolonged in the rectum than in the colon and that the rectal response is mediated by cholinergic as well as non-cholinergic mechanisms. They further suggest that vasoactive intestinal polypeptide (VIP) is likely to be the neurotransmitter responsible for the latter component.

Previous studies of the pelvic neural control of motility in the rectum and colon (Fasth, Hultén & Nordgren, 1980) have indicated that these nerves convey excitatory as well as inhibitory influences to the rectum, whereas they are solely excitatory in the colon, and these findings were confirmed in the present study. It therefore seems that the parasympathetic neuro-effector organization in these two regions of the large intestine differ both with regard to the control of blood flow and motility.

Some of these results have been reported previously in preliminary form (Andersson, Edwards, Järhult & Mellander, 1982).

METHODS

Animals

The experiments were performed on adult cats. Food but not water was withheld for at least 14 h before each experiment. The animals were anaesthetized i.v. with chloralose (50 mg/kg) and urethane (100 mg/kg). After anaesthesia a tracheal cannula was inserted to facilitate spontaneous respiration.

Surgical and experimental procedures

Pelvic nerve preparation. The abdominal cavity was exposed by a mid-line incision. The pelvic nerves were dissected free as they emerged from the sacral roots, divided and mounted in platinum ring electrodes for subsequent stimulation of the peripheral ends. Square wave impulses (8 V, 5 ms) were delivered from a Grass stimulator (S 88). In all experiments the sympathetic nerves to the large intestine were cut.

Rectum preparation. The large intestine was divided at the site where it enters the pelvic cavity, which is the borderline between colon and rectum according to the terminology of Langley & Anderson (1895). The luminal content was expelled and the cut ends of the gut ligated. A flaccid water-filled balloon for subsequent motility recordings was inserted through, and anchored by ligatures around, the anus. After heparinization (750 i.u./kg) a polythene shunt circuit, which incorporated a photo-electric drop counter, was inserted between the right femoral artery and the inferior mesenteric artery to permit direct recordings of rectal blood flow. All branches of the inferior mesenteric artery except those supplying the rectum were ligated. Thus the recorded arterial blood flow was restricted to that which supplied the rectum and this was verified at the end of the experiment by observing the tissue distribution of Indian ink injected into the shunt. The vein running along the mesenteric border of the rectum was cannulated and the venous effluent returned to the animal via a funnel connected to the right jugular vein. Rectal venous outflow pressure was kept constant at about 5 mmHg. All tributaries of this vein other than those draining the rectum were ligated to ensure that the effluent blood was exclusively rectal. Samples of rectal effluent blood were collected at intervals via a T-tube in this venous shunt adjacent to the rectum for determinations of haematocrit, VIP, and substance P. Intra-arterial infusions were made via the arterial shunt.

Colon preparation. A small incision was made in the caecum and the luminal content of the colon removed. A water-filled balloon for subsequent colonic motility recordings was introduced through the incision into the proximal colon and anchored by ligatures around the incision. The colonic vein was cannulated at its proximal end and the venous effluent diverted through a photo-electric drop-counter and returned to the animal via a funnel connected to the right jugular vein. Colonic venous outflow pressure was kept constant at about 5 mmHg. The colonic vein was ligated at the borderline between colon and rectum and venous tributaries from other tissues than colon were ligated to ensure that the effluent blood was exclusively colonic.

In experiments in which drugs were administered i.a. to the colon, an arterial shunt circuit was inserted between the right femoral artery and the colonic branch of the superior mesenteric artery. The inferior mesenteric artery was then ligated; a procedure which we have found has no effect on blood flow in the colon, in agreement with previous observations (Hultén, 1969).

Combined rectum and colon preparation. In order to make a simultaneous comparative study of the effects of vasoactive agents on both the colon and rectum it was necessary to ensure that they were administered to both viscera at identical arterial concentrations. For this purpose, both the colon and rectum were supplied with blood from the right femoral artery via a common shunt with distal Y-tube connections to the colonic branch of the superior mesenteric artery and the inferior mesenteric artery, respectively. The connexion between the colonic artery and the inferior mesenteric artery was ligated where the latter bifurcated immediately adjacent to the gut wall and a loose ligature was tied around the colon at this level, care being taken not to injure branches of the pelvic nerves. By this means the large intestine was separated into a proximal, vascularly isolated, colonic preparation and a distal, vascularly isolated, preparation consisting mainly of the rectum and of a 2-3 cm segment of the distal colon, the whole distal segment being designated 'rectal preparation'. Blood flow was recorded in the colonic and rectal branches of the arterial shunt with drop-recorders, and vasoactive substances were administered into the proximal common shunt. Balloons for motility recordings were installed as described above. This combined colon-rectum preparation was also used for simultaneous studies of rectal and colonic blood flow and motor effects of pelvic nerve stimulation.

Recordings. Arterial blood pressure was monitored continuously from a catheter in the right brachial artery by means of a Statham P 23 transducer. Rectal and colonic blood flows were recorded continuously by means of the drop-recorders operating ordinate writers. Rectal and colonic motility was monitored from the water-filled balloons connected to water reservoirs attached to Grass force displacement transducers, FT 10 C (see Grände, Järhult & Mellander, 1974). With this arrangement motility was recorded in terms of volume change at constant pressure (15 cm H₂O). Arterial pressure, blood flow, and motility were recorded on a Grass Polygraph.

Protocols. After the preparatory surgery the animals were left to equilibrate for about 30 min.

In one group of animals, rectal and colonic blood flow and motor responses to pelvic nerve stimulation for 2-3 min were investigated. The recovery period between stimulations was invariably sufficient to allow blood flow and motility to return to control levels. The impulses were delivered either continuously (1, 2, 4, 8 or 16 Hz), or in 1 s bursts (10, 20, 40, 80 or 160 Hz) at 10 s intervals.

These two patterns of stimulation are designated 'continuous stimulation' and 'stimulation in bursts', and the comparative results relate to observations where the total number of impulses/min was identical. The sequence in which different patterns and frequencies of stimulation were tested was randomized. Up to fifteen such stimulations were carried out in each experiment.

In a second group of animals, arterial and rectal venous blood samples were collected for determination of VIP and substance P before, during and after a 3 min period of stimulation, applied either at 4 Hz continuously, or in 1 s bursts (40 Hz), at 10 s intervals, the total number of impulses/min being the same. In each animal, two to four such tests were performed, separated by a resting period of 30 min, and the sequence was randomized.

In a third group of animals, VIP and substance P were administered by close i.a. infusion to the colon and/or the rectum while simultaneous recordings of blood flow and motility were made. The effects of these peptides were compared with those of pelvic nerve stimulation in the same animals.

In each of these three groups of animals, observations were made before and after cholinergic blockade (atropine 1 mg/kg).

Drugs

Atropine (1 mg/kg), hexamethonium (10 mg) and propranolol (Inderal, I.C.I.; 0.25 mg/kg) were administered as intravenous injections 15 min prior to such experiments in which muscarinic, ganglionic, or β -adrenergic blockade was desired. In order to produce α -adrenergic blockade, phentolamine (CIBA) was employed and given as a continuous i.v. infusion (0.15 mg/min) starting 15 min before and maintained throughout the experimental period. Aprotinin (Trasylol, Bayer; 300 K.I.U./min) was continuously infused close arterially to block kinin formation. VIP (obtained from Dr V. Mutt, Stockholm) and substance P (Sigma), dissolved in 0.9% saline containing 1% albumin were administered by close arterial infusion at rates of 0.1–0.3 ml/min.

Radioimmunoassay

Samples of arterial, rectal venous, and colonic venous blood were collected in ice-chilled test tubes containing aprotinin (1000 K.I.U./ml blood) for determination of VIP and substance P. The samples were immediately centrifuged at +4 °C, and the plasma samples stored at –20 °C until assayed. VIP and substance P concentrations were measured by radioimmunoassay (Mitchell & Bloom, 1978; Bloom & Long, 1982).

Calculations and statistics

Vascular resistance was calculated as perfusion pressure divided by blood flow. Blood flow was expressed in ml 100 g⁻¹ (wet wt.) min⁻¹. The output of VIP and substance P was calculated as the veno-arterial difference in plasma concentration multiplied by the plasma flow (estimated from haematocrit determinations) and expressed as pmol 100 g⁻¹ min⁻¹. The increase in arterial plasma VIP and substance P concentrations during peptide infusions was calculated from data for infused amount of the peptide and control plasma flow. The arterial 'dead space time delay' during peptide infusion was estimated at the end of the experiment by infusion of Indian ink into the arterial shunt, measuring the time from the start of an infusion to first appearance of ink in the tissue at the relevant blood flows. Rectal and colonic contractile responses to graded nerve stimulation were expressed as a percentage of the maximum obtained, in the absence of atropine, in each experiment, because the balloon method does not permit a direct comparison in absolute figures of the responses in the two viscera.

Statistical analyses were based on the Student's *t* test, and *P* values less than 0.05 were considered to be significant. Data are expressed as mean values $\pm$ s.e. of means.

RESULTS

Rectal and colonic blood flow 'at rest'

Under steady-state 'resting' conditions at normal arterial pressure ($\approx$ 120 mmHg) and with cut autonomic nerves, rectal blood flow averaged 46 ± 2 ml 100 g⁻¹ min⁻¹, which was roughly twice that in the colon (25 ± 2 ml 100 g⁻¹ min⁻¹; *P* < 0.001).

Neither of the values was much affected by changes in blood pressure in the range 80–170 mmHg, indicating good autoregulation of blood flow in both regions of the large intestine.

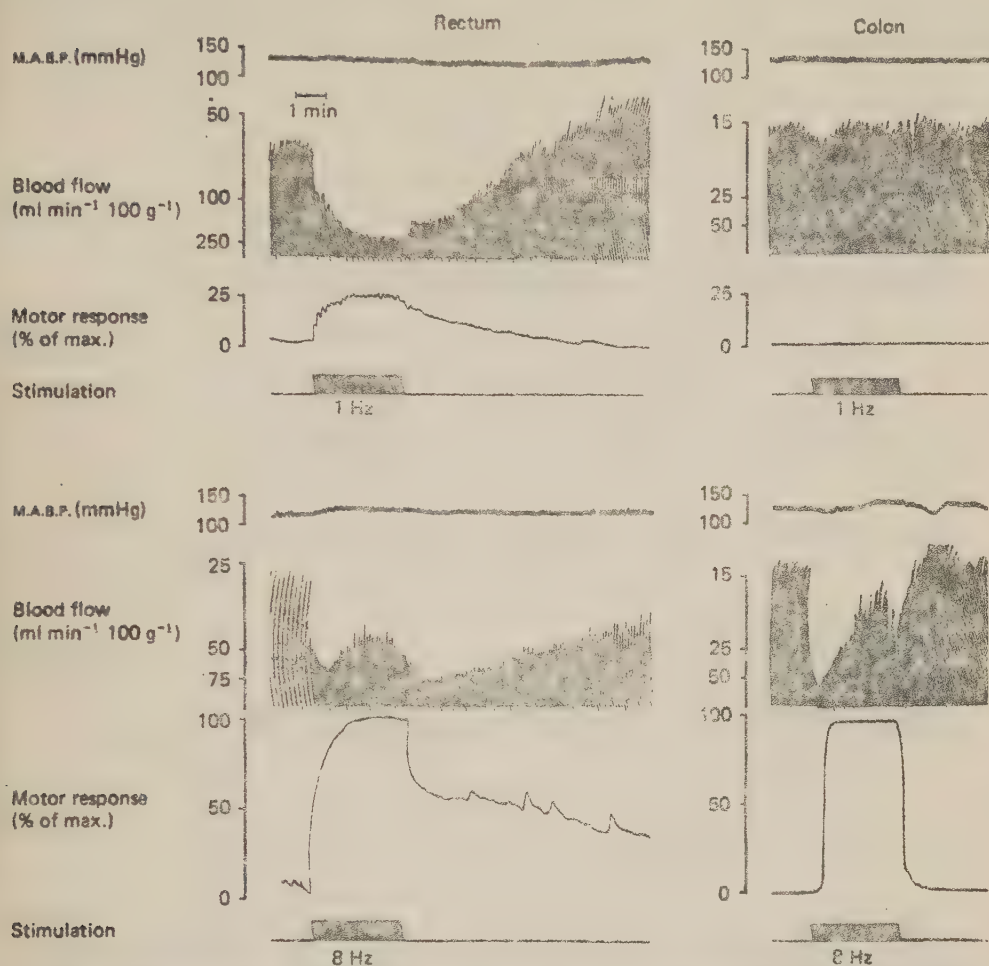


Fig. 1. Effects on mean arterial blood pressure (M.A.B.P.), blood flow and motor responses in the rectum (left panels) and colon (right panels) of continuous stimulation of the pelvic nerves for 3 min at 1 Hz (upper panels) and 8 Hz (lower panels). Data for rectum and colon taken from different experiments.

Vascular and motor responses to pelvic nerve stimulation

This series of experiments describes effects of pelvic nerve stimulation in the absence of pharmacological blockade.

Continuous stimulation. Typical recordings of rectal and colonic blood flow and motor responses to continuous stimulation for 3 min at 1 and 8 Hz are shown in Fig. 1. In the rectum, stimulation at 1 Hz (upper left panel) evoked a very marked vasodilatation which stabilized within 1–2 min and was maintained throughout the

period of stimulation. Consequently, there was a clear-cut contractile motor response. When stimulation was discontinued the dilator response waned very gradually. This pronounced dilator response in the rectum is in marked contrast to the small, transient dilatation seen in the colon under the same conditions (upper right panel). Stimulation at 1 Hz caused no detectable contractile motor response in the colon. Stimulation at 8 Hz (lower panels) evoked pronounced vasodilator responses

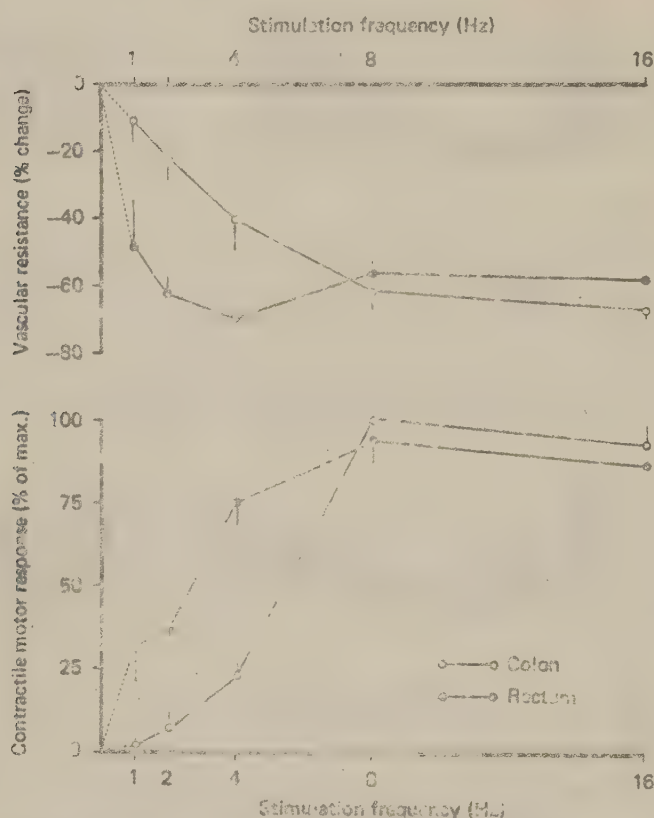


Fig. 2. Frequency-response curves showing the vascular resistance, tone and contractile motor response (peak values) in the rectum ($\square$; $n = 4$) and colon ($\circ$; $n = 4$) to graded continuous stimulation of the pelvic nerves for 3 min. The magnitudes of the contractile motor responses in this and the following figures were estimated from a common base line neglecting spontaneously occurring rhythmic contractions in the unstimulated situation.

in both the rectum and colon, but they tended to fade during maintained stimulation. This effect was due, at least in part, to mechanical interference with the blood flow by the concomitant increased (maximal) contractile motor responses. The colonic vasodilator and motor responses disappeared quickly after the 3 min period of stimulation whereas the rectal effects faded much more gradually (over about 10 min, Fig. 1).

It thus appears that there is a more effective and more prolonged pelvic neural control of the resistance vessels in the rectum than colon. Maximal dilator effects in

the rectum were evoked by continuous stimulation at 2–4 Hz, corresponding to blood flow values of 200–250 ml 100 g⁻¹ min⁻¹ at normal perfusion pressure. In the colon, stimulation at 8–16 Hz was required to obtain maximal dilatation, and peak flow rates rarely exceeded 150 ml 100 g⁻¹ min⁻¹.

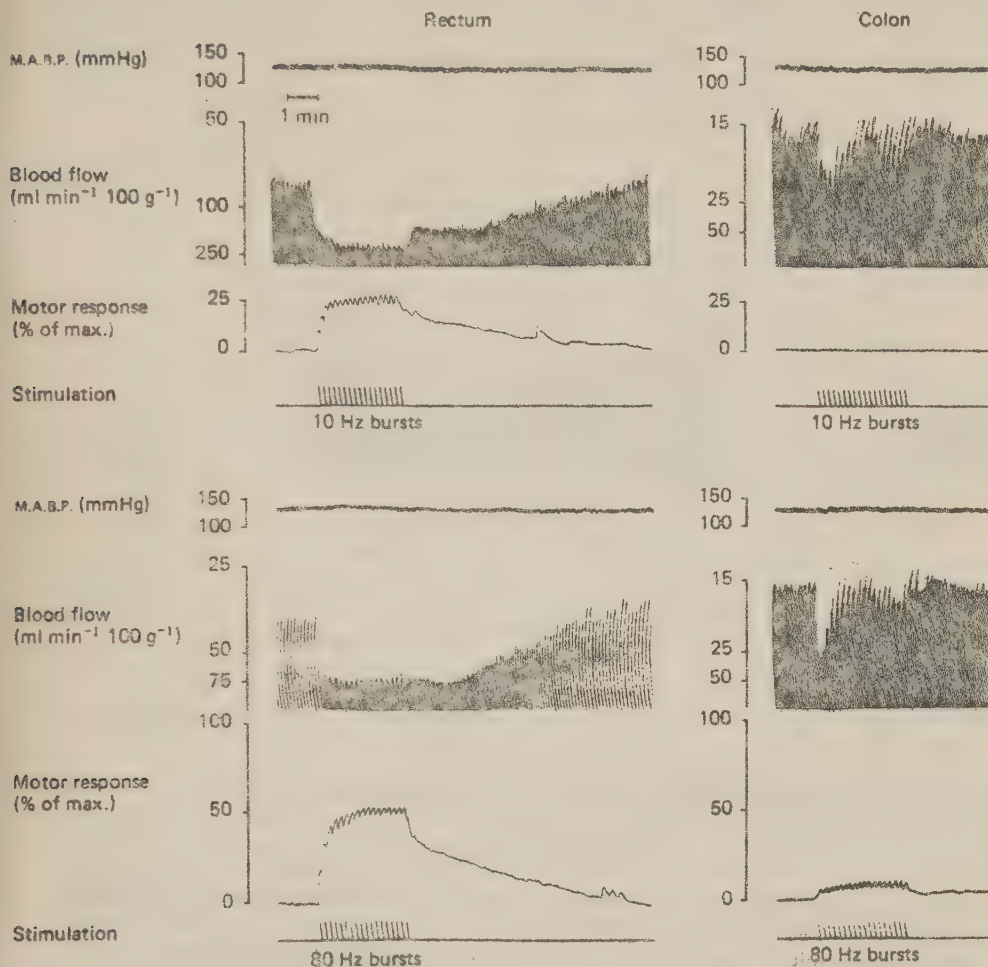


Fig. 3. Effects on mean arterial blood pressure, blood flow and motor responses in the rectum (left panels) and colon (right panels) of pelvic nerve stimulation in 1 s bursts at 10 s intervals for 3 min at frequencies of 10 Hz (upper panels) and at 80 Hz (lower panels). Observations made on the same animals as in Fig. 1.

Mention should be made that in some rectal preparations the neural dilator response was less stable than shown in Fig. 1, but it was never abolished during the period of stimulation, as was the case in the colon.

Fig. 2 summarizes the results of all the experiments in this series in terms of frequency-response curves for the rectal and colonic vasodilator and contractile motor responses to continuous stimulation. The data relating to the decrease in

vascular resistance refer to the peak increase in blood flow during stimulation (which should be recalled was short-lasting in the colon). Note that the curves for both the dilator and motor responses in the rectum are displaced to the left of those in the colon, indicating that the pelvic neural control of the rectum is mainly effected by low-frequency, and that of the colon by high-frequency, continuous stimulation.

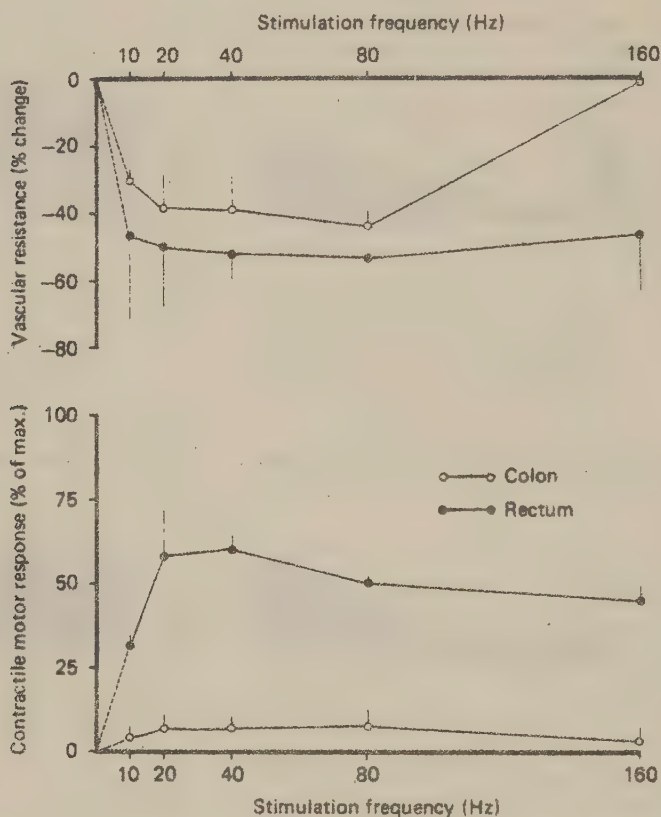


Fig. 4. Frequency-response curves showing the vascular resistance response and contractile motor response (peak values) in the rectum (●; $n = 3$) and colon (○; $n = 6$) to pelvic nerve stimulation in 1 s bursts at 10 s intervals for 3 min at different frequencies.

Stimulation in bursts. The effects of continuous stimulation (1–16 Hz) were compared with those of high-frequency stimulation (10–160 Hz) in bursts of 1 s duration applied at 10 s intervals, so that in the case of each comparison the same total number of impulses were delivered in unit time. This type of stimulation in bursts mimics a common pattern of discharge in single pelvic nerve fibres *in vivo* (de Groat *et al.* 1982). Original records of the responses to stimulation in bursts (10 and 80 Hz) are shown in Fig. 3. Stimulation in bursts produced quite similar vasodilator response patterns in both the rectum and the colon to those during continuous stimulation at the corresponding frequencies (cf. Figs. 1 and 3). The rectal contractile motor response to stimulation in bursts was also quite similar although it was less forceful during stimulation at 80 Hz in bursts than 8 Hz continuously. This may well explain

why there were no signs of mechanical interference with flow during stimulation in bursts at 80 Hz. In the colon, neither stimulation in bursts at 10 Hz nor continuous stimulation at 1 Hz evoked any detectable contractile response. Stimulation at 80 Hz in bursts caused a very small contraction by comparison with that during stimulation

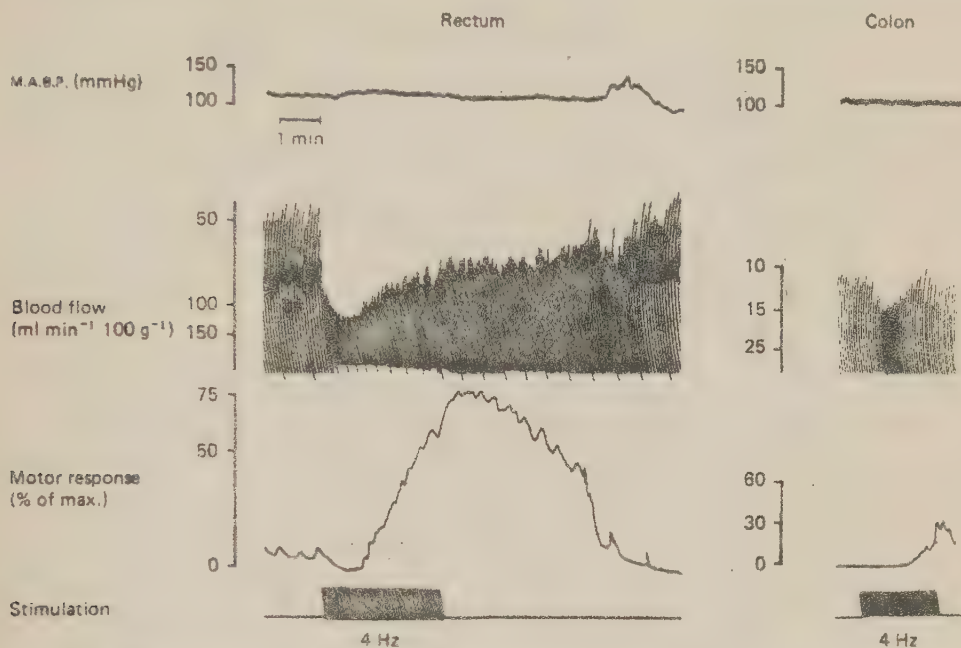


Fig. 5. Effects, after muscarinic blockade (atropine 1 mg/kg), on mean arterial blood pressure, blood flow and motor responses in the rectum (left panel) and the colon (right panel) of continuous stimulation of the pelvic nerves at 4 Hz. Data for the colon are reproduced for ease of comparison from Andersson, Bloom & Järhult (1983), by permission.

at 8 Hz continuously (Figs. 1 and 3). The transient nature of the concomitant blood flow response in the colon during stimulation in bursts at 80 Hz shows that this autoregulatory phenomenon cannot be ascribed merely to mechanical interference with the flow of blood.

Fig. 4 summarizes the results of stimulation in bursts at different frequencies. The maximal rectal and colonic vasodilator responses (upper panel) were somewhat less pronounced than those evoked by the corresponding continuous stimulation (cf. Fig. 2), but the frequency-response curves were steeper at stimulation in bursts, an almost maximal dilatation occurring at 10 Hz. Burst stimulation evoked clear-cut contractile motor responses in the rectum (Fig. 4, lower panels) and the maximum represented about 60% of that observed during continuous stimulation (Fig. 2). In contrast, stimulation in bursts produced virtually no colonic contractile response.

Vascular and motor responses to pelvic nerve stimulation in the presence of atropine

Continuous stimulation. Fig. 5 illustrates typical vascular and motor changes in the rectum and colon in response to continuous stimulation at 4 Hz after muscarinic blockade. Pelvic nerve stimulation still caused substantial vasodilator responses in both regions of the large intestine which, with respect to magnitude and time course, approximated to those recorded in the absence of atropine. The only difference was

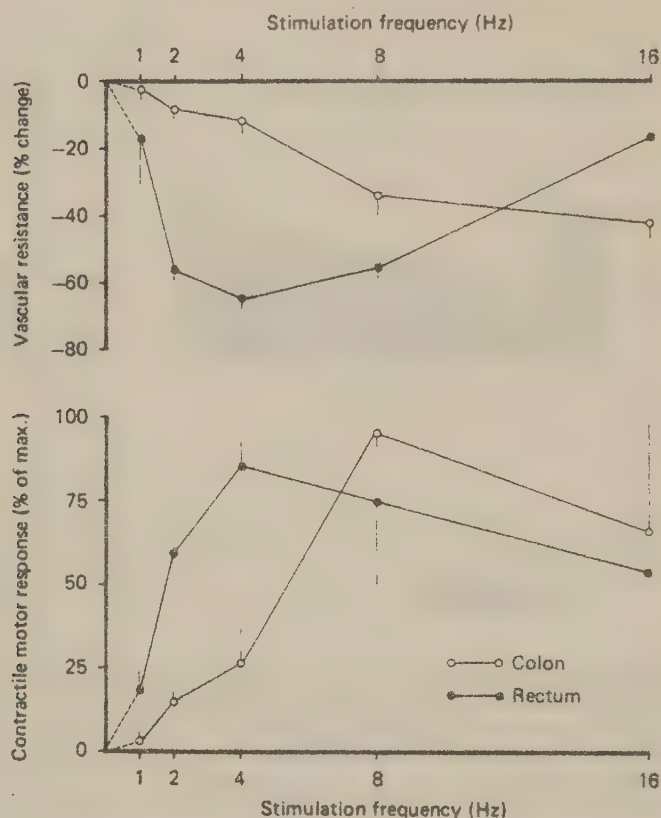


Fig. 6. Frequency-response curves showing peak vascular resistance and contractile motor responses (including the post-stimulatory motor response) in the rectum (●; $n = 4$) and colon (○; $n = 6$) to continuous stimulation of the pelvic nerves at different frequencies in atropinized cats (1 mg/kg). The contractile motor responses are expressed as a percentage of the maximum obtained in the absence of atropine. The colonic data are reproduced from Andersson *et al.* (1983), by permission.

that, following an early peak, the rectal vasodilator response regularly diminished, but it was never abolished during the period of stimulation, as was the case in the colon. The rectal motor response after muscarinic blockade consisted of a small but consistent initial relaxation followed by a secondary gradual contraction. Immediately after the period of stimulation a reinforcement of the contraction was regularly found to precede the relaxation (Fig. 5). In the colon stimulation invariably caused a pure

contraction, but this was always delayed and the delay was inversely related to stimulus frequency.

The frequency-response curves for both the vasodilator and motor contractile effects (the latter including the post-stimulatory excitation) of stimulation under these conditions in the rectum and colon are shown in Fig. 6. The contractile motor responses are expressed as a percentage of the maximum obtained in the absence of

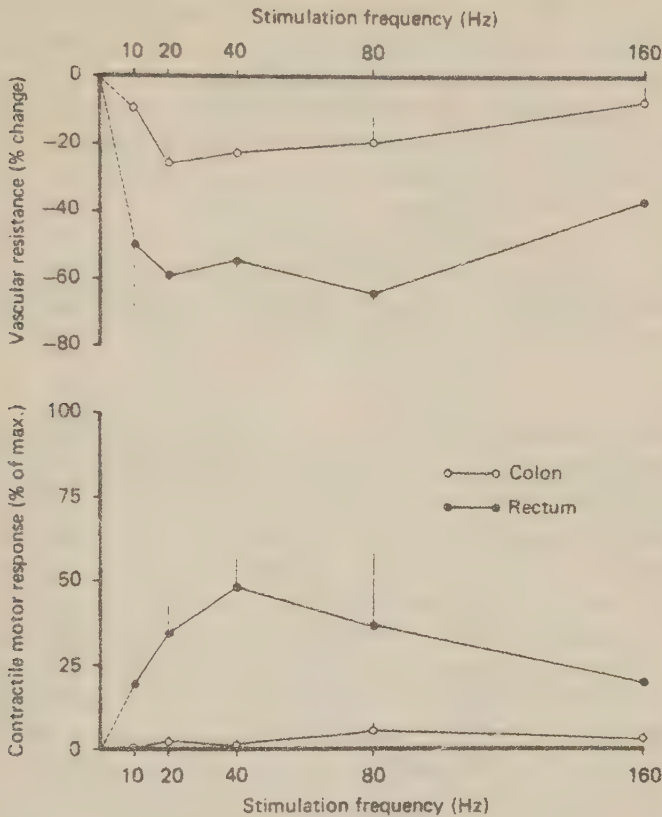


Fig. 7. Frequency-response curves showing peak vascular resistance and contractile motor responses (including the post-stimulatory motor response) in the rectum (●; $n = 4$) and colon (○; $n = 6$) to pelvic nerve stimulation in 1 s bursts at 10 s intervals at different stimulus frequencies in atropinized cats (1 mg/kg). The contractile motor responses are expressed as in Fig. 6. The colonic data are reproduced from Andersson *et al.* (1983).

atropine. The results show that both the vascular and the contractile motor responses in the rectum are more sensitive to continuous stimulation at low frequencies than the responses in the colon.

Stimulation in bursts. After muscarinic blockade, the rectal and colonic blood flow and motor response patterns to stimulation in bursts at 40 Hz were similar to those caused by continuous stimulation at 4 Hz (cf. Fig. 5), except that stimulation in bursts caused no contractile motor response in the colon.

The frequency-response relations obtained in this series of experiments are shown

in Fig. 7. Comparison of the data in Figs. 6 and 7 shows that stimulation in bursts caused similar vasodilator responses in both the rectum and the colon to those which occur during continuous stimulation. The contractile motor responses to the former pattern of excitation were somewhat smaller in the rectum and almost non-existent in the colon.

Pharmacological studies

Ganglionic blockade, which was achieved by pre-treatment with hexamethonium (10 mg i.v.), completely abolished all rectal and colonic vascular and motor responses to stimulation of the pelvic nerves, showing that the site of neural stimulation in these experiments was preganglionic. The responses to pelvic nerve stimulation were not affected by α -adrenergic blockade by phentolamine (i.v. infusion at 0.15 mg/min) or by β -adrenergic blockade by propranolol (0.25 mg/kg i.v.), indicating that none of these effects was mediated by adrenergic mechanisms; nor were they affected by intra-arterial infusion of aprotinin in amounts (300 K.I.U./min) calculated to raise local plasma aprotinin concentration to a level sufficient to inhibit local production of bradykinin from kallikrein (e.g. Fasth, Hultén, Nordgren & Zeitlin, 1981). This indicates that the neural responses reported here cannot be related to bradykinin formation.

Output of VIP and substance P from the rectum in response to pelvic nerve stimulation

The mean outputs of VIP and substance P from the rectum (calculated as veno-arterial difference in plasma concentration $\times$ plasma flow) before, during and after a 3 min period of stimulation at 4 Hz continuously, and in 40 Hz bursts at 10 s intervals, are illustrated in Fig. 8A (lower graphs); the associated changes in rectal vascular resistance and motility are shown in the upper graphs of the Figure. Corresponding data after muscarinic blockade are shown in Fig. 8B. It is noteworthy that stimulation led to a marked and rapid increase in VIP output, the magnitude and time course of which correlated well with the vasodilator response, but not with the motor response (Fig. 8A). Both the output of VIP and the vasodilator response were greater during continuous than during burst stimulation. The output of VIP in response to pelvic nerve stimulation was reduced after atropine (Fig. 8B), suggesting that a muscarinic mechanism might contribute to its release. However, the possibility cannot be ruled out that the somewhat larger rise in blood flow which occurred before the administration of atropine in these particular experiments contributed to this effect by 'washing' VIP out of the tissue more efficiently. The output of VIP from the rectum in response to pelvic nerve stimulation was greater per unit weight than that we have previously reported in respect of the colon (Andersson, Bloom & Järhult, 1983).

There was no significant increase in the output of substance P from the rectum in these experiments (Fig. 8A and B, right lower graphs).

Effect of VIP and substance P infusions

In the experiments described above, the peak VIP concentration in the rectal venous effluent plasma averaged 190 ± 20 pm at 4 Hz continuous stimulation after muscarinic blockade. Fig. 9 (right) shows the effect of a close i.a. infusion of VIP on

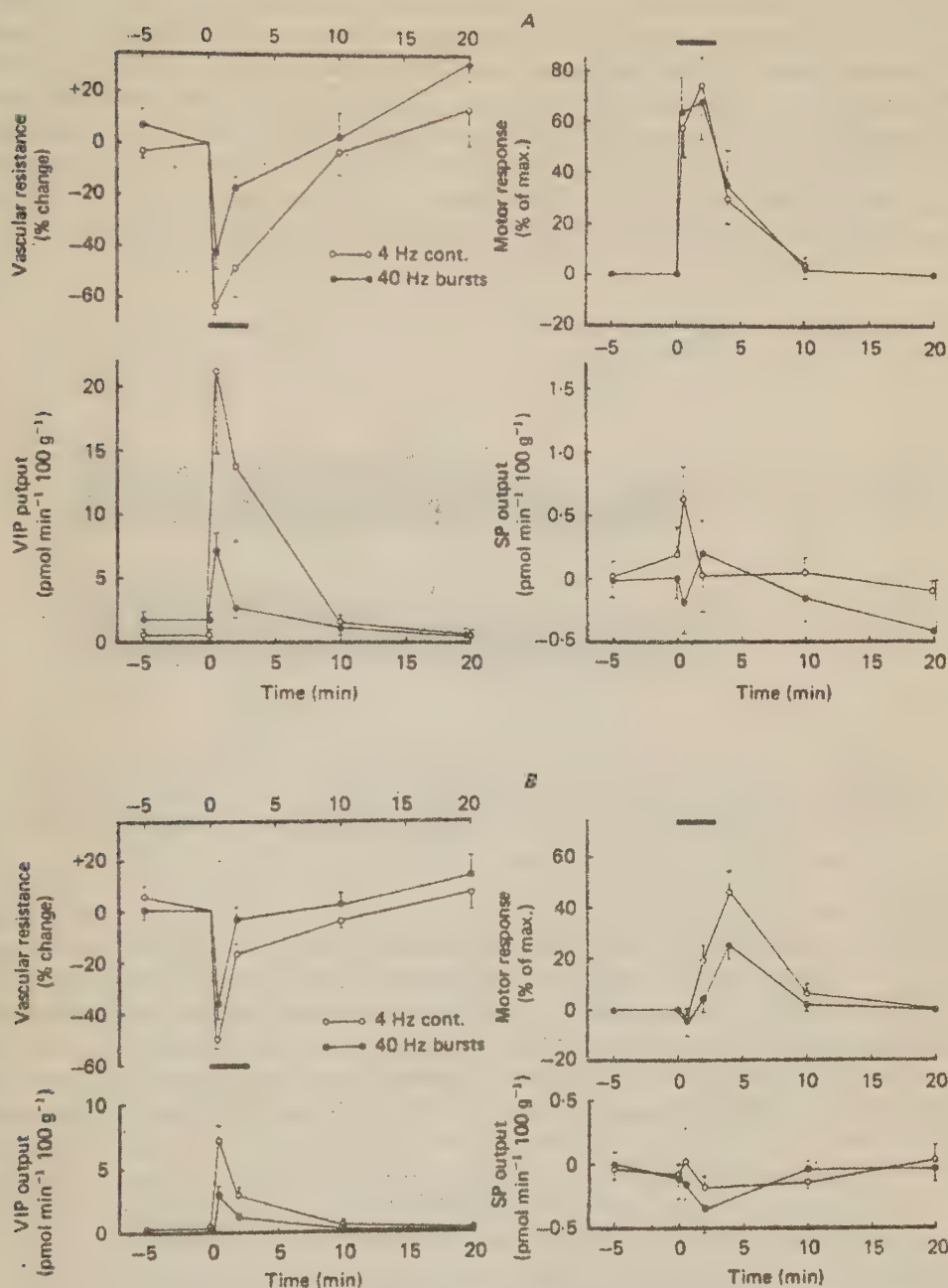


Fig. 8. Rectal vascular resistance and motor responses (upper graphs) and VIP and substance P (SP) outputs (lower graphs) from the rectum in response to pelvic nerve stimulation for 3 min (horizontal bars), continuously at 4 Hz ($\circ$; $n = 7$) or in 1 s bursts (40 Hz) at 10 s intervals ($\bullet$; $n = 5$). A: results obtained in the absence of atropine. B: results obtained in the presence of atropine (1 mg/kg).

simultaneously recorded rectal and colonic blood flows. The dose administered (which was calculated to increase the arterial plasma concentration of VIP by 25 nM) raised the recorded peak VIP plasma concentration in the local venous effluent to about 200 pM, i.e. to about the same level as that during stimulation in atropinized cats. This dose of VIP induced a marked vasodilatation in the rectum, the magnitude and time course of which were similar to those evoked by stimulation at 4 Hz continuously under the same conditions (Fig. 9). This low dose of VIP had no effect on colonic blood flow; nor did it produce any discernible effect on either rectal or colonic motility.

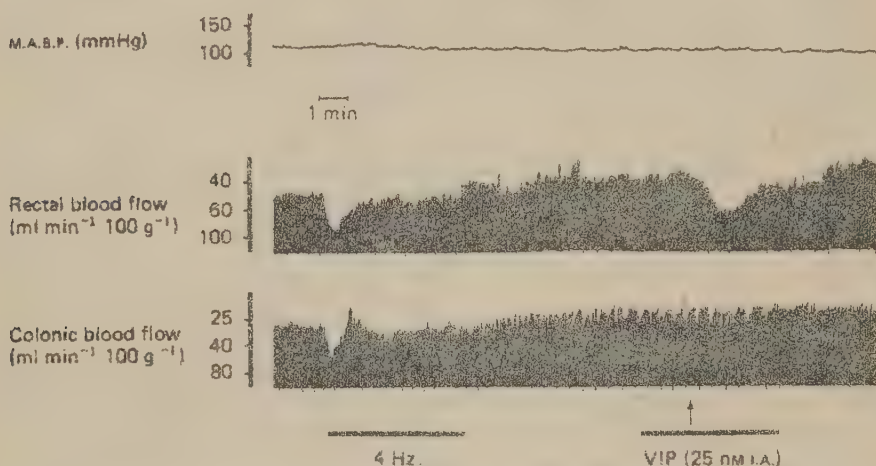


Fig. 9. Effects, after muscarinic blockade, on mean arterial blood pressure, rectal and colonic blood flow of continuous pelvic nerve stimulation at 4 Hz for 5 min (left) and close I.A. infusion of VIP for 5 min in a dose calculated to increase arterial plasma VIP concentration by 25 nM (right). The arrow shows the time at which VIP reached the tissues.

Close I.A. infusions of VIP in higher concentrations caused dose-dependent vasodilator responses in the rectum which matched the maximum responses to pelvic nerve stimulation. High doses of VIP also caused colonic vasodilatation. Fig. 10 shows dose-response curves for the vasodilatation which occurred in both viscera in a representative experiment, in which VIP was infused simultaneously into the combined colon-rectum preparation at graded arterial concentrations. The displacement of the rectal dose-response curve to the left of that for the colon indicates a 50- to 100-fold greater sensitivity of the rectal than the colonic resistance vessels to this peptide. The threshold concentration of VIP which elicited colonic vasodilatation in this experiment was about 50 nM, a concentration capable of evoking roughly maximal rectal dilatation (Fig. 10).

Intra-arterial infusions of VIP, even in very high concentrations ($> 1 \mu\text{M}$), failed to evoke any motor responses in either the rectum or the colon in any of these experiments.

The concentrations of substance P in arterial and rectal venous effluent plasma in the control state were both about 50 pM and were not significantly altered by pelvic nerve stimulation. The effects of close I.A. infusions of substance P were tested in doses

which raised the local venous concentration up to about 200 pM. This peptide evoked a transient (< 1 min) vasodilator and a maintained contractile motor response in both the colon and the rectum. Vascular resistance decreased maximally by about 50% in both regions and this occurred at as low an arterial concentration as 1 nM. The dilator response preceded the contractile motor response by 30–60 s and the latter was more closely dose-dependent in the range of doses tested, with a threshold

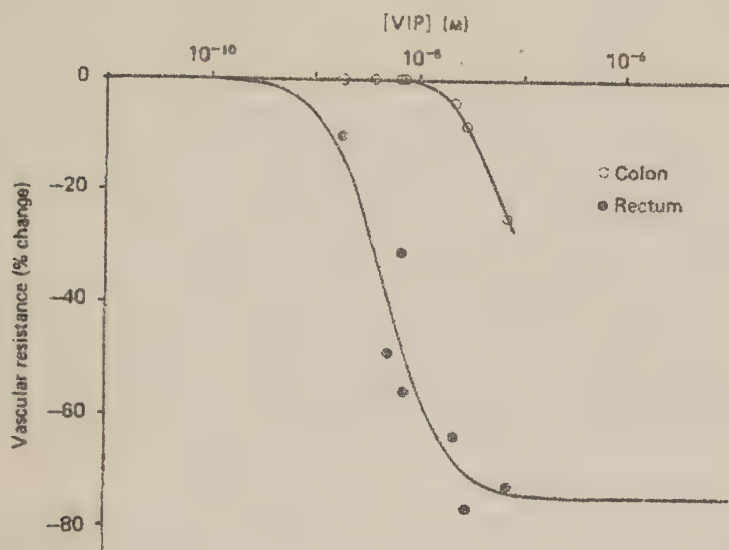


Fig. 10. Dose-response curves from a representative experiment showing rectal (●) and colonic (○) vascular resistance responses to close i.a. infusions of VIP. Doses refer to estimated increases in arterial plasma VIP concentration.

somewhat lower in the rectum (about 0.1 nM) than in the colon (about 1 nM). The contractile motor responses in colon and rectum, and possibly also the colonic dilator response, to substance P infusions resembled those evoked by pelvic nerve stimulation in atropinized cats with respect to the time course. However, substance P failed to mimic the non-cholinergic vasodilator response to neural stimulation in the rectum. It must be emphasized, however, that the effects of substance P infusions that are described here were only obtained when the concentration of the peptide in the venous effluent plasma far exceeded that ever observed during stimulation of the pelvic nerves.

DISCUSSION

The present study is the first, so far as we are aware, which attempts to provide a quantitative analysis of the rectal vascular response to pelvic nerve stimulation and compare that directly with corresponding events in the colon. The results indicate that the vasodilator control by the pelvic nerves is more pronounced in the rectum than in the colon in the cat. These observations, together with observed different neural motor responses of these viscera, indicate a partly different parasympathetic neuro-effector organization in the two regions of the large intestine. The finding that

the basal blood flow in the rectum is about twice that in the colon suggests that the rectum is more densely vascularized, or has a higher basal metabolic rate, than the colon in this species.

Possibly the most distinct difference which was revealed in the course of this study was that the rectal vasodilatation was maintained throughout the period of pelvic nerve stimulation and then returned to the control level very gradually, whereas in the colon the response was much more transient, fading away early while stimulation was continuing. This dissimilarity was observed both with continuous low-frequency and intermittent high-frequency stimulation. These two stimulus patterns were chosen to mimic the discharge *in vivo* as recently recorded from single sacral parasympathetic neurones in this species (de Groat *et al.* 1982). These authors found that some neurones discharged continuously at low frequencies (1–4 Hz), whereas others fired in short intermittent bursts at frequencies up to 60 Hz. The present results show that vascular smooth muscle effectors are highly responsive to stimulation in bursts at these high frequencies (Figs. 3 and 4), previously considered to be supraphysiological (cf. Folkow, 1952). These findings are in agreement with recent studies of neurogenic vascular reactivity in the submaxillary and parotid glands (Andersson, Bloom, Edwards & Järhult, 1982; Andersson, Bloom & Edwards, 1982) and skeletal muscle (Andersson, 1983). However, different tissues seem to have different optimum frequencies. Using continuous stimulation, the rectal vessels are effectively controlled at low frequencies, with maximum dilatation at 2–4 Hz, whereas the maximal colonic response is observed at 8–16 Hz.

After muscarinic blockade, pelvic nerve stimulation still caused vasodilatation in both the rectum and colon, but in the latter it became more evanescent, in agreement with Hultén's previous report (1969). On the other hand, the non-cholinergic rectal vasodilatation persisted throughout the period of stimulation, although it was somewhat attenuated after atropine. Thus, the non-cholinergic pelvic neural dilator response was also more pronounced in the rectum than colon.

Pelvic nerve stimulation, either continuously or in bursts, elicited pronounced contractions in the rectum in the absence of atropine. In the colon, however, only continuous stimulation caused consistent contractile responses, and no significant motor response to stimulation in bursts could be elicited at any frequency. The reason for this 'uncoupling' effect in the colon is not known, but it may well be due to failure of ganglionic transmission during preganglionic excitation in bursts, as suggested by the finding that pronounced colonic contractions can be evoked *in vitro* in response to transmural stimulation in high-frequency bursts (P. O. Andersson, unpublished observations).

Further evidence for a different parasympathetic neuro-effector organization in these two regions of the large intestine is provided by the fact that stimulation after muscarinic blockade still caused an excitatory, albeit delayed, motor response in the colon whereas the rectum relaxed, as has also previously been reported by others (Fasth *et al.* 1980). In the present study it was found that this initial rectal relaxation was invariably followed by a secondary gradual contractile response during prolonged stimulation. Apparently, a non-cholinergic contractile response to pelvic nerve stimulation is present in both the colon and the rectum, but preceded by a relaxation in the latter.

The results of this study tend to refute the possibility that the non-cholinergic rectal vasodilatation is mediated by catecholamines, bradykinin formation, or substance P, but provided the following evidence that it is mediated by VIP. This peptide, which is known to be present in nerve fibres in the vicinity of blood vessels in the large intestine (e.g. Buffa, Capella, Fontana, Usellini & Solcia, 1978; P.-O. Andersson & R. Uddman, to be published), was released from the rectum in response to stimulation of the pelvic nerves in amounts, and with a time course, which correlated very well with the concomitant rectal vasodilator response. The output of VIP, expressed per unit tissue weight, was also greater than in most other organs where VIP has been implicated as a neurotransmitter, with the possible exception of the submaxillary gland (Bloom & Edwards, 1980); the rectal output of VIP exceeded that reported for other regions of the gastrointestinal tract (Fahrenkrug, Haglund, Jodal, Lundgren, Olbe & Schaffalitzky de Muckadell, 1978; Andersson *et al.* 1983). Moreover, i.a. infusion of VIP, which mimicked the increase in the peptide plasma concentration in the rectal venous effluent during stimulation, evoked a rectal vasodilator response of similar magnitude and time course to that elicited by nerve stimulation. VIP infusions evoked dose-dependent rectal vasodilatations which could be as large as the maximal neural response. Infusions of VIP to the rectum and colon simultaneously indicated that the rectal vessels were some 50–100 times more sensitive to the peptide than the colonic vessels, and the doses of VIP required to elicit colonic vasodilatation were of the same high order or magnitude as those reported previously by Eklund, Jodal, Lundgren & Sjöqvist (1979).

Thus, there is strong evidence for suggesting that VIP is a neurotransmitter in the rectum and that its release is responsible for rectal atropine-resistant vasodilatation. Although the possibility that this peptide is also involved in the colonic vasodilatation cannot be excluded, VIP release from the colon during stimulation was much less than that from the rectum and it was poorly correlated to the colonic vasodilator response (Andersson *et al.* 1983). The fact that the rectal output of VIP during stimulation was twice as great before than it was after muscarinic blockade (cf. Figs. 9 and 10) suggests that a muscarinic mechanism may possibly contribute to its release.

It has been suggested that VIP mediates the atropine-resistant contractile motor response to pelvic nerve stimulation in the colon (Eklund *et al.* 1979), but our data do not support this hypothesis, since i.a. infusions of the peptide in doses $> 1 \mu\text{M}$ caused no discernible motor effect in either the colon or rectum.

Since there was no significant increase in substance P output during stimulation and since the vasodilator response to infusion of this agent showed little resemblance to the neural response it seems less likely that this peptide mediates the atropine-resistant rectal vasodilator response. However, substance P infusions evoked contractile motor responses in both the colon (cf. Hellström & Rosell, 1981) and the rectum, of some resemblance to the atropine-resistant responses to pelvic nerve stimulation, which possibly could be claimed as evidence of its involvement in the neural motor control of these viscera.

The functional significance of the rectal vasodilator response to pelvic nerve stimulation is unknown. The mucosa may be the main site where the rectal dilatation occurs (cf. Langley & Anderson, 1895), as seems to be the case in the colon (Hultén,

1969). Preliminary experiments in our laboratory have indicated that, with intact pelvic nerves, the response can be induced reflexly by mechanical distension of the rectum or the anal sphincter and, hence, might fulfil some function associated with defaecation, or be linked to other events such as secretion. Interestingly, a pronounced mucosal dilatation in the rectum has been observed in response to emotional stimuli, indicated by the report (see Davenport, 1961) that a male medical student who had volunteered for rectoscopic examination, on discovering that a female student was peering through the instrument, developed a marked 'blushing' reaction of his rectal mucosa.

It is a pleasure to acknowledge the skilled technical assistance of Mrs Anne Björkly, Mrs Tora Jinde and Mr Gerry McGregor. The study was supported by grants from the Swedish Medical Research Council (2210), the British Medical Research Council, The British Science and Engineering Research Council, the Medical Faculty, University of Lund, Sweden, and Dr P. Håkanson's Foundation.

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HAEMODYNAMICS OF PELVIC NERVE INDUCED PENILE ERECTION IN THE DOG:
POSSIBLE MEDIATION BY VASOACTIVE INTESTINAL POLYPEPTIDE

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SUMMARY

1. The haemodynamics of erection were elucidated in the anaesthetized dog by analysing in quantitative terms the changes of penile arterial inflow, venous outflow and tissue volume during graded pelvic nerve stimulation. The study also provides information on possible neurotransmitter mechanisms of the erectile response.

2. Erection evoked by pelvic nerve stimulation appeared to result from two main circulatory events: First, there was a prompt dilatation of the penile 'resistance vessels' causing a greatly increased arterial inflow which in the early phase bypassed the cavernous bodies and, hence, increased venous outflow to the same extent. Second, the erectile response proper began after a distinct delay (≈ 20 s). This was apparently caused by sudden opening of low resistant 'shunt vessels' diverting part of the arterial inflow into the cavernous bodies leading to rapid filling. During the filling phase arterial inflow greatly exceeded venous outflow, and returned to the venous outflow level again in the steady state of full erection. The initial dilator response seemed to ensure rapid erection by establishing a high pressure head from the arterial microvessels to the cavernous spaces.

3. The threshold frequency for the penile vasodilator response to pelvic nerve stimulation was 1-2 Hz and was always higher for the erectile volume response, *viz.* 2-4 Hz. Maximal effects for both were obtained at 16 Hz, causing on the average a 25-fold increase in peak arterial inflow, a 17-fold increase in venous outflow and a 107% increase in penile volume.

4. Muscarinic blockade by atropine caused no significant decrease in the pelvic nerve induced blood flow response, but clearly curtailed the erectile response. This indicates that the dilatation of the penile

2.

'resistance vessels' is mainly non-cholinergic in nature, whereas a cholinergic mechanism seems to contribute to the erectile volume response proper.

5. Pelvic nerve stimulation caused a substantial output of vasoactive intestinal polypeptide (VIP) from the penis which was correlated in onset and duration to the vasodilator response. Intra-arterial (i.a.) infusion of VIP elicited moderate erection, and a penile vasodilator response which resembled the neural response. Similar effects were evoked by i.a. infusion of substance P, but the output of this peptide from the penis during stimulation was poorly correlated to the vascular events. These in vivo observations indicate that VIP might be the neurotransmitter responsible for the non-cholinergic pelvic nervous penile vasodilatation.

INTRODUCTION

Much research has been devoted to clarifying the autonomic nervous control of penile erection and there seems to be quite general agreement that the response is mainly mediated via the pelvic nerves (for ref. see e.g. Bell, 1972). Pharmacological evidence indicates that the pelvic nervous control of penile vasodilatation during erection is mainly non-cholinergic in nature (Henderson & Roepke, 1933; Dorr & Brody, 1967), but the neurotransmitter has not yet been adequately defined.

Several hypotheses for the haemodynamics of penile erection have been presented in the literature, largely based on anatomical and angiographic studies in various species including man and on flow studies in cadavers (for ref. see Christensen, 1954; Newman & Northup, 1981) but also on a few more functional haemodynamic studies in experimental animals in vivo (e.g. Henderson & Roepke, 1933; Dorr & Brody, 1967; Sjöstrand & Klinge, 1979). The most common opinion, at present, seems to be that the erectile volume increase is mainly accomplished via penile arterial vasodilatation, and that a constrictor mechanism on the venous side is of little or no importance for cavernous filling (Dorr & Brody, 1967; Sjöstrand & Klinge, 1979; Newman & Northup, 1981).

In the previous haemodynamic investigations, neural penile vasodilatation was estimated from change of arterial inflow pressure at constant flow perfusion, or from change of venous outflow, and the erectile response was derived from recordings of cavernous tissue pressure, or sometimes followed volumetrically. Only occasionally, however, was more than one of these parameters recorded simultaneously,

but this seems to be required for more detailed understanding of the complex haemodynamics of penile erection. In fact there is need of simultaneous quantitative data on both arterial inflow, venous outflow and penile volume change during erection, and the pelvic neural frequency-response characteristics should be defined. Such analyses were performed in the present study in the dog.

An attempt was also made to define the non-cholinergic neurotransmitter mechanism of penile erection. Previous morphological demonstrations of nerve structures containing VIP (Larsson, Fahrenkrug & Schaffalitzky de Muckadell, 1977; Polak, Mina, Gu & Bloom, 1981) and substance P (Alm, Alumets, Brodin, Håkanson, Nilsson, Sjöberg & Sundler, 1978) in the vicinity of vascular smooth muscle in the penis hint at their possible involvement in penile erection. This possibility gained some indirect support, with regard to VIP, from the finding of an inhibitory effect of this peptide on rhythmic activity in cavernous smooth muscle in vitro (Willis, Ottesen, Wagner, Sundler & Fahrenkrug, 1981). So far as we are aware, there are still no functional studies in vivo which corroborate this hypothesis. In this study an attempt was made to bridge this gap by analysing in vivo the penile vascular effector responses to close arterial infusions of VIP and substance P, by determining the output of these peptides from the penis during pelvic nerve induced erection, and by correlating the peptide release to the erectile vascular events.

Some of the present results have been reported in preliminary form (Mellander, Andersson & Bloom, 1984).

METHODS

The experiments were performed on male adult beagle and mongrel dogs (mean weight 28 ± 2 kg). Food but not water was withheld for at least 14h before each experiment. The animals were anaesthetized intravenously with pentobarbital sodium (30 mg/kg bwt). After anaesthesia, a tracheal cannula was inserted to facilitate spontaneous respiration.

Surgical and experimental procedures

Pelvic nerve preparation. The abdominal cavity was exposed by a mid-line incision. The pelvic nerve fibres to the penis were dissected free bilaterally, divided and mounted in platinum ring electrodes for subsequent stimulation of the peripheral ends. Square wave impulses (10 V, 5 ms), found to cause supramaximal nerve excitation, were delivered from a Grass stimulator (S 88). In all experiments the sympathetic chain fibres and the hypogastric nerve fibres were left intact.

Penile preparation. The penis was dissected free of skin except for the prepuce, and the superficial veins of the prepuce at the abdominal wall were ligated and divided. The penis was then reflected caudally. After heparinization (750 IU/kg), the two dorsal veins of the penis were cannulated about 1 cm from the pubic bone. The two cannulas were connected via a Y-tube to a common shunt, diverting penile venous outflow through a photo-electric drop-counter and blood was then returned to the animal via a funnel connected to the right jugular vein. The venous blood flow, recorded at an outflow pressure of 1-2 mmHg, can be considered to represent total venous drainage from the penis distal to the site of cannulation, since an external occlusion pressure well above vein pressure applied by a snare around the proximal penis, caused no increase in recorded venous outflow. Erectile responses of the glans penis were recorded volumetrically by placing it inside a transparent cylindrical plethysmograph, sealed proximally by the prepuce which was pulled over the exterior of the cylinder and fixed by a surrounding ligature. By this arrangement the whole glans was kept in fixed position permitting reliable erectile volume change recordings, unaffected by accidental movements or activity of the retractor penis muscle. The plethysmograph was filled with isotonic saline and the temperature was kept constant at 37° C. In experiments in which close arterial infusions to the penis were made, a Y-tube shunt circuit was inserted between the right femoral artery and the two dorsal penile arteries. The soft tissue weight of the penis (penile bone and skin excluded) was determined at the end of the experiments. The weight of the penis distal to the point of flow cannulation averaged 25 ± 3 g and that of the glans 14 ± 2 g.

Recordings. Arterial blood pressure was monitored continuously from a catheter in the right brachial artery by means of a Statham P 23 AC transducer. Penile venous outflow was recorded continuously by means of the drop-recorder operating an ordinate writer. The calibration of the flow recording system was checked frequently during the course of each experimental manoeuvre by measuring flow simultaneously with a graduated cylinder. Samples of penile effluent blood were collected at intervals via the venous shunt for determinations of haematocrit, VIP and substance P. Penile volume changes were monitored from the plethysmograph connected to a fluid reservoir, in turn attached to a Grass force displacement transducer, FT 10C (see Grände, Järhult & Mellander, 1974). Arterial pressure, penile venous outflow and penile volume change were recorded on a Grass Polygraph.

Protocols. After the preparatory surgery, the animals were left to equilibrate for about 30 min.

In one series of experiments the effects of frequency-graded pelvic nerve stimulations on penile blood flow and erectile volume responses were investigated. The impulses were delivered in the range from 0.25 to 20 Hz and the stimulations were separated by a resting period of 15 min.

In a second series of experiments penile haemodynamic responses were observed during simultaneous repetitive collection of arterial and penile venous blood samples for determinations of VIP and substance P before, during and after a 3 min period of pelvic nerve stimulation applied at 20 Hz. These stimulations were separated by a resting period of 30 min.

In a third series of experiments penile hemodynamic responses to close arterial infusions of acetylcholine, VIP, substance P and papaverine were recorded.

Drugs

Atropine (1 mg/kg bwt) and phenoxybenzamine (15 µg/kg bwt) were administered i.v. 15 min prior to experiments for muscarinic or α-adrenergic blockade. Close arterial infusions (0.1 - 0.3 ml/min) were performed of the following: Papaverine (0.4 mg/min), acetylcholine (10-50 µg/min), VIP (obtained from Dr. V. Mutt, Stockholm; 1 µg/min) and substance P (Sigma; 1 µg/min). The first two agents were dissolved in 0.9 % saline and the latter two in saline containing 1 % albumin.

Radioimmunoassay

Samples of arterial and penile venous blood were collected in ice-chilled test tubes containing aprotinin (1000 K.I.U./ml blood; Bayer) for determinations of VIP and substance P. The samples were immediately centrifuged at +40°C, and the plasma samples stored at -20°C until assayed. VIP and substance P concentrations were measured by radioimmunoassay (Mitchell & Bloom, 1978; Bloom & Long, 1982; McGregor & Bloom, 1983).

Calculations and statistics

The rate of penile arterial inflow of blood during erection/detumescence was calculated as venous outflow ($\text{ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$) plus/minus the simultaneously recorded rate of erectile volume change ($\text{ml blood} \times \text{min}^{-1} \times 100\text{g}^{-1}$). The unit weight (100 g) refers to penile soft tissue. VIP and substance P output was calculated as the veno-arterial plasma concentration difference multiplied by the plasma flow (estimated from haematocrit determinations) and expressed as $\text{fmol} \times \text{min}^{-1} \times 100\text{g tissue}^{-1}$. Vascular resistance was calculated as perfusion pressure divided by penile venous outflow of blood.

Statistical analyses were based on the Student's *t*-test and *P* values less than 0.05 were considered to be significant. Data are expressed as mean values $\pm$ SEM.

RESULTS

Penile blood flow and volume in the flaccid control state.

With severed pelvic nerves, but otherwise intact autonomic innervation, penile venous blood flow in the control state averaged $15 \pm 5 \text{ ml} \times \text{min}^{-1} \times 100\text{g tissue}^{-1}$ in 6 dogs at a mean arterial inflow pressure of $130 \pm 5 \text{ mmHg}$. Control vascular resistance thus averaged about 9 PRU ($\text{mmHg} \times \text{ml}^{-1} \times \text{min} \times 100\text{g tissue}$) signifying the presence of a marked resting tone in the penile resistance vessels. This tone was only slightly depressed by adrenergic blockade (phenoxybenzamine, 15 µg/kg bwt, i.v.), but very markedly inhibited by i.a. infusion of the smooth muscle relaxing agent papaverine (0.4 mg/min) indicating that resting tone was mainly intrinsic (myogenic) in nature. Penile volume was very stable in the control state in all dogs implying that there were no

significant spontaneous alterations of blood volume in the cavernous tissue and, hence, that arterial inflow of blood equalled the recorded venous outflow.

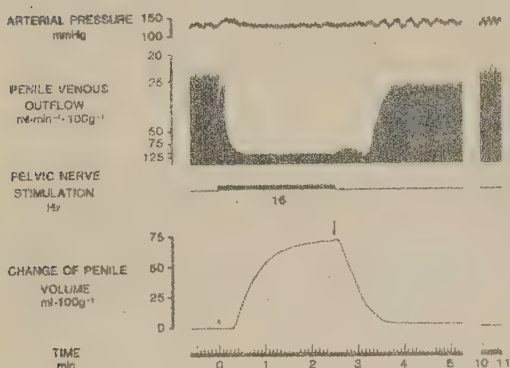


Fig. 1. Effects on arterial pressure, penile venous outflow, and penile volume during erection in the dog evoked by optimum stimulation of the pelvic nerves (16 Hz). Arrows denote start and end of stimulation. Note the prompt onset of the penile blood flow increase and the delayed start of the erectile volume increase.

Penile haemodynamic responses to pelvic nerve stimulation

A typical pattern of response to bilateral pelvic nerve stimulation (10 V, 5ms) at optimum frequency (16 Hz, see below), observed in the absence of atropine, is illustrated in Fig. 1. Nerve stimulation caused an almost instantaneous penile vasodilator response (delay <5 s) leading, after a transient biphasic flow pattern to an increase in venous outflow from the resting value of 23 to a maximal value of $110 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$ in 40 s. This dilatation was then maintained throughout the stimulation period. Penile volume (lower panel) was unaffected during the very early phase of this vasodilator response, but after a delay of 20 s nerve stimulation caused an abrupt increase in volume. At this time, blood flow had reached the level of $85 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$. The rate of penile volume increase was very fast initially ($90 \text{ ml} \times \text{min}^{-1} \times 100\text{g} \text{ tissue}^{-1}$) and then more gradual. Penile volume approached maximum (increment $70 \text{ ml}/100\text{g} \text{ tissue}$) at the end of the 2.5 min stimulation period. The tumescence of the penis was apparent also in the marked increase in penile length, circumference, and turgidity, and appeared to be a maximal erectile response under these experimental conditions. Cessation of nerve stimulation led to a rapid disappearance of the erectile response as evidenced visually and by the quick decline in recorded penile volume which approached the pre-stimulatory control value

in about 1 min (Fig. 1). Venous outflow showed a biphasic pattern (decrease/increase) during the period of penile detumescence and then a rapid, followed by a slower, decrease back to the control level. The fact that penile volume after erection so promptly returned to the pre-stimulatory control value in this and all other experiments strongly indicates that the erectile volume increase is caused by accumulation of blood in the intravascular (cavernous) compartment with no significant transcapillary fluid loss into the extravascular space, despite high microvascular pressure. This, in turn, suggests that the increased flow during erection mainly bypasses a conventional capillary network.

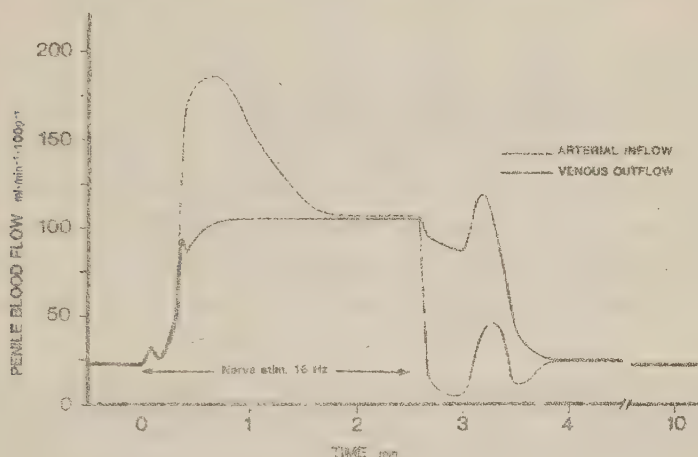


Fig. 2. Changes of penile arterial inflow and penile venous outflow during erection in the dog evoked by optimum stimulation of the pelvic nerves (16 Hz). Data taken from the experiment depicted in Fig. 1. Arterial inflow calculated from venous outflow values and concomitantly observed rates of penile volume change. Arterial inflow exceeds venous outflow during the phase of erectile cavernous filling and is lower than venous outflow during the phase of penile detumescence.

The penile arterial inflow was derived quantitatively from the recorded venous outflow and the rates of penile volume change during erection/detumescence, in turn caused by pooling/emptying of blood in the cavernous tissue. Such flow data, calculated from the experiment in Fig. 1, are illustrated in Fig. 2 which also shows a replotted version of the original venous outflow curve. It can be seen that during pelvic nerve stimulation arterial inflow was identical to venous outflow up to the flow rate of $85 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$, i.e. until the onset of penile erection. During the phase of cavernous filling, however, arterial inflow greatly exceeded venous outflow, during the transient peak (Fig. 2) almost by a factor of 2. After this peak, arterial inflow declined again

and approached venous outflow during the steady state of full erection. Cessation of pelvic nerve stimulation led to quick penile detumescence and, concomitantly, to a very abrupt and marked decrease in arterial inflow followed by a transient oscillatory flow pattern before flow stabilized at the pre-stimulatory control level. The decrease in venous outflow was much smaller and more delayed during the period of detumescence due to the addition of blood from the emptying cavernous tissue, after which it quickly returned to control.

These results are interpreted to indicate two main haemodynamic events in erection, an early dilator response of the penile 'resistance vessels' and a subsequent opening of 'shunt vessels' diverting flow into the cavernous spaces (see Discussion). Relevant for such an interpretation were some additional findings. Mechanical occlusion of the venous outflow caused no increase in penile volume in the flaccid control state, but a clearcut one when applied in the phase of submaximal erection indicating no significant forward flow into the cavernous bodies in the control state due to closed 'shunt vessels'. Venous effluent blood in the flaccid state had low oxygen content in the control state but became highly oxygenated during erection as evidenced by a drastic change in blood colour.

The effects of frequency-graded pelvic nerve stimulations on penile venous blood flow and penile volume are illustrated in Fig. 3. The stimulations were supramaximal (10 V, 5 ms) and performed at 15 min intervals. Excitation at low rates (0.25, 0.5, and 1 Hz) caused in this dog no discernible vasodilatation or erectile volume increase whatsoever. Stimulation at 2 Hz evoked a clearcut vasodilator response, increasing venous outflow from 41 to 65 $\text{ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$, but still no erectile response. At 4 Hz venous blood flow increased to a value of 93 $\text{ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$ and this was associated with an erectile response starting at the time blood flow exceeded the value of about 85 $\text{ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$. Both the vasodilator and erectile responses were larger at 8 Hz, and they became maximal at 16 Hz with a blood flow increase to 120 $\text{ml} \times \text{min}^{-1} \times 100 \text{ g tissue}^{-1}$ and a volume increase by 73 $\text{ml}/100 \text{ g tissue}$, both responses being well maintained during the period of stimulation. Excitation at 20 Hz caused an increase in venous outflow to a peak value of 105 $\text{ml} \times \text{min}^{-1} \times 100 \text{ g}^{-1}$ which gradually declined somewhat to about 95 $\text{ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$. This dilator response was associated with an initial rapid volume increase (note increased paper speed in this record), but this erectile response became only about half as large as that at 16 Hz.

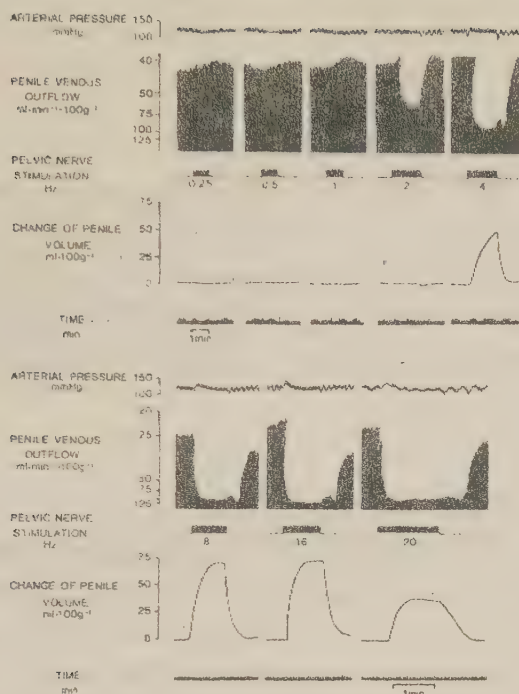


Fig. 3. Effects on arterial pressure, penile venous outflow and penile volume in the dog evoked by graded pelvic nerve stimulation (0.25-20 Hz). The threshold frequency for the blood flow response was 2 Hz and for the erectile volume increase 4 Hz. Both responses became maximal at 16 Hz and declined at 20 Hz. (Note increased paper speed at 20 Hz stimulation.)

and it was not well maintained during the period of stimulation. This impairment of the erectile response was not due to a 'fatigue' phenomenon of repetitive stimulation, since maximal effects were regained at a stimulation at 16 Hz performed 15 min later, *viz.* the experiment depicted in Fig. 1.

It is concluded that an optimum erectile response in the dog was evoked at a pelvic nerve excitation rate of about 16 Hz, and that the erection was smaller both at higher and lower frequencies, the very low ones being ineffective. The consistent delay in these experiments between the blood flow response and the erectile response might indicate partly different neurotransmitter mechanisms for these two responses. This was further supported by their different threshold frequencies and by the fact that at 8 and 20 Hz blood flow increased to roughly the same levels, but the erectile response was clearly larger at 8 than 20 Hz (Fig. 3).

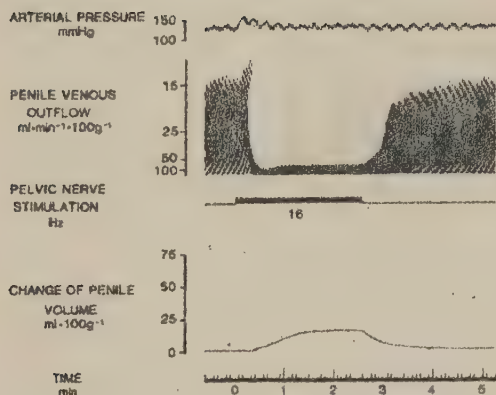


Fig. 4. Effects on arterial pressure, penile venous outflow and penile volume of pelvic nerve stimulation (16 Hz) in the presence of atropine (1 mg/kg) observed in the same dog as used in Fig. 1. Comparison of Figs. 1 and 4 shows that atropine markedly depressed the erectile volume response but not the blood flow response.

In Fig. 4 are illustrated the effects on penile venous outflow and penile volume of pelvic nerve stimulation at 16 Hz in the presence of atropine (1 mg/kg bwt, i.v.) which may be compared with the corresponding effects observed in the absence of atropine in the same dog (Fig. 1). It can be seen that in the atropinized dog nerve stimulation still caused a pronounced vasodilator response which increased venous outflow to $100 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$ in the initial phase, later maintained at the level of about $90 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$. Penile volume, as in Fig. 1, started to rise when blood flow surpassed the level of about $85 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$, but in this case the penile volume increase occurred at a much slower rate and the final volume increase was only 22% of the maximal one obtained in the absence of atropine. Consequently, calculated arterial inflow was smaller after atropinization, comprising $120 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$ during the peak (cf. Fig. 2). Upon cessation of the stimulation venous outflow and penile volume returned to the control levels.

It may be concluded from these experiments that the main pelvic nervous mechanism for dilatation of the penile 'resistance vessels' is noncholinergic in nature, whereas the rapid redistribution of arterial flow and the accumulation of blood in the cavernous tissue, i.e. the erectile response, at least partly, is dependent upon a cholinergic mechanism.

This conclusion, and the principal findings described in the foregoing which refer to the same dog, were largely confirmed in the other animals in which graded pelvic nerve stimulation was performed in the absence and

presence of atropine. Stimulation at 16 Hz was found to evoke maximal penile dilator and erectile responses also in the other dogs, although there were quite marked individual differences with regard to magnitude of the maximal responses, as shown in Table 1. On the average, such nerve excitation in the absence of atropine caused a 17-fold increase in venous outflow, a 25-fold increase in peak arterial inflow and a 107% increase in penile volume. Atropine reduced the neural erectile penile volume increase from 107 to 41%, a difference which was significant ($p < 0.05$). The average venous and arterial flow values during stimulation in the presence of atropine also tended to be somewhat lower but the difference was not statistically significant.

Table 1. Penile haemodynamic data at rest and during pelvic nerve stimulation at 16 Hz in the absence and presence of atropine (1 mg/kg bwt, i.v.). Data, obtained from 3 dogs, are expressed as mean values $\pm$ SEM; range within parenthesis.

	No atropine (n=5)	Atropine (n=4)	Significance
Resting penile blood flow (ml·min ⁻¹ ·100g ⁻¹)	14.2 $\pm$ 5.2 (4.5–22.4)	9.3 $\pm$ 2.9 (3.6–13.5)	N.S.
<u>Nerve stimulation:</u>			
Maximal venous outflow (ml·min ⁻¹ ·100g ⁻¹)	237 $\pm$ 59 (120–450)	188 $\pm$ 48 (102–266)	N.S.
Peak arterial inflow (ml·min ⁻¹ ·100g ⁻¹)	344 $\pm$ 102 (207–607)	218 $\pm$ 57 (120–312)	N.S.
Maximal penile volume increase (ml·100g ⁻¹)	107 $\pm$ 54 (37–213)	41 $\pm$ 24 (16–89)	$p < 0.05$

The threshold frequency for evoking a pelvic nervous penile vasodilator response varied between 1 and 2 Hz in the whole material and the threshold frequency for producing an erectile volume increase was always 1–2 Hz higher. The frequency-response characteristics in the absence and presence of atropine are shown in Fig. 5 in which the increase in penile venous outflow and in penile volume are expressed in per cent of the maximal ones observed in each dog in the absence of atropine. The blood flow and erectile responses in the absence of

atropine were clearly graded in relation to the excitation rate up to 16 Hz. Atropinization did not significantly affect the flow response but markedly depressed the erectile response. Both responses were always significantly reduced by raising the excitation rate from 16 to 20 Hz. Since the effects at 20 Hz reached no steady state but gradually declined during stimulation these data are not included in Fig. 5.

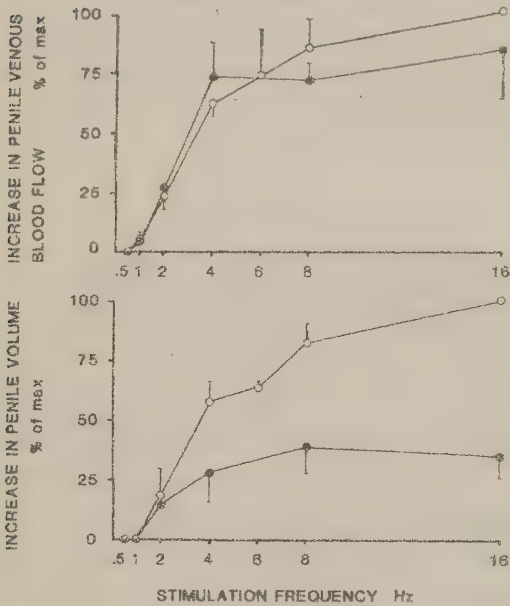


Fig. 5. Frequency-response curves depicting steady state increases in penile venous outflow and penile volume in the dog in response to graded pelvic nerve stimulations in the absence (o) and presence (●) of atropine (1 mg/kg). Responses expressed in % of the maximal ones obtained in each experiment in the absence of atropine.

The data in Fig. 5 further point to fact that both cholinergic and non-cholinergic mechanisms contribute to the penile haemodynamic responses during pelvic nerve excitation, and suggest that the cholinergic transmitter may be of special significance for the erectile volume response proper. This was further supported by effects observed during infusion of acetylcholine into the two dorsal arteries of the penis. In the dose range tested (10-50 $\mu\text{g}/\text{min}$), acetylcholine evoked a dosedependent increase in penile venous outflow (in the highest dose an about five-fold increase) and a clear-cut, though submaximal, erectile response (volume increase 11 ml/100g tissue; cf. Table 1). The latter showed time characteristics which were similar to those during pelvic nerve stimulation whereas the vasodilator response appeared to be more sluggish than the neural flow response. These findings, however, should merely be taken as a demonstration that acetylcholine is capable of simulating qualitatively the neural penile erectile response. A

quantitative comparison with the neural response is difficult to make since acetylcholine infused into the dorsal arteries is probably distributed differently within the penile vascular bed than during neural release and its access to the smooth muscle effectors might be limited due to the poorly developed capillary network in the erectile tissue.

VIP and substance P in relation to penile erection

As mentioned in Introduction there is some indirect support from morphological and in vitro observations for suggesting VIP and substance P as putative neurotransmitters involved in penile erection, a hypothesis tested below by making some functional observations on these peptides in vivo.

VIP. Infusions of VIP into the two dorsal arteries of the penis in a dose of 1 $\mu\text{g}/\text{min}$ produced an about fivefold increase in penile venous outflow of blood and a clearcut, though submaximal, erectile response (volume increase 9 ml/100g tissue; cf. Table 1). The development of the blood flow response showed quite similar time characteristics to those during nerve stimulation in the presence of atropine and the effect was well maintained throughout the period of VIP infusion, after which vascular tone recovered gradually. The onset of the erectile response to VIP appeared to be more sluggish than the neural response. The quantitative comparison between the response to VIP and pelvic nerve stimulation of course is open to the same reservations as stated for the acetylcholine infusion experiments.

Determinations of the release of VIP into the penile blood during pelvic nerve stimulation were performed at the stimulation frequency of 20 Hz, a rate known to cause optimum neural VIP release in another parasympathetically controlled tissue, viz. the submaxillary gland (Bloom & Edwards, 1980; Andersson, Bloom, Edwards & Järhult, 1982). In the control state there was no detectable spontaneous output of VIP from the penile tissue, but pelvic nerve stimulation consistently caused a release of this peptide. The extent to which this occurred in the absence of atropine is shown in Fig. 6 in which the VIP output ($\text{fmol} \times \text{min}^{-1} \times 100\text{g tissue}^{-1}$) was calculated as the veno-arterial plasma concentration difference times venous effluent plasma flow (mean values $\pm$ SEM from 8 experiments). The lower panel of the figure shows the concomitant penile vasodilator response. It can be seen that pelvic nerve stimulation caused a rapid and pronounced output of VIP, coordinated in time with the penile vasodilator response, and a rapid decrease in the output upon cessation

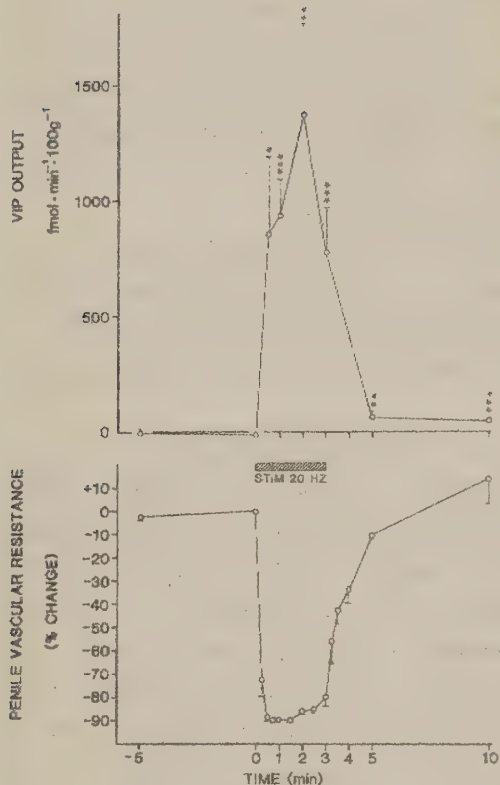


Fig. 6. Output of VIP from the canine penis and change of penile vascular resistance in response to stimulation of the pelvic nerves (20 Hz) in the absence of atropine. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

of the stimulation. At the peak, the VIP output comprised $1370 \pm 390 \text{ fmol} \times \text{min}^{-1} \times 100\text{g}^{-1}$. It is possible that the promptness of the VIP release into the blood is underestimated in Fig. 6 since the peptide quite likely is distributed into the entire arterial inflow which in the early phase of erection much exceeds the venous outflow (cf. Fig. 2), and the calculation was based on the latter. Calculation of VIP output as veno-arterial concentration difference times arterial plasma flow indicated that the VIP output was more than 80% of maximum already in the first sample 30 s after the onset of nerve stimulation. The VIP data presented in Fig. 6, as stated, were obtained during stimulation in the absence of atropine, but corresponding analysis in the presence of this drug showed no significant difference in the results. These experiments provide functional evidence in vivo for suggesting VIP as a neurotransmitter of the penile 'resistance vessel' dilatation.

Substance P. Infusion of substance P into the dorsal arteries of the penis in a dose of $1 \mu\text{g}/\text{min}$ evoked an about tenfold increase in penile venous outflow of blood and a clearcut, though submaximal, erectile

response (volume increase 11 ml/100g tissue; cf. Table 1) with time characteristics quite similar to those observed during pelvic nerve stimulation in the presence of atropine. Determination of substance P in arterial and penile venous plasma were performed in the same experiments as described in Fig. 6. In the control state there were clearly detectable concentrations of this peptide in both the arterial and venous blood but no indication of a spontaneous substance P output from the penis. Pelvic nerve stimulation (20 Hz) caused no change of the substance P concentration in the arterial blood but venous concentration altered, although not in a consistent manner: It rose in 5 out of 8 stimulation experiments and dropped in the others, and the nerve induced output of this peptide from the penis was thus highly variable. Occasionally, nerve stimulation caused quite a pronounced substance P output giving an average value for the whole material of $3070 \pm 1460 \text{ fmol} \times \text{min}^{-1} \times 100\text{g}^{-1}$ ($p < 0.05$) in the sample taken 30 s after the onset of stimulation. In no case, however, was the substance P output maintained during the period of penile vasodilatation and erection. Such poor correlation to penile haemodynamics questions a role of this peptide in the pelvic nerve induced penile erection.

DISCUSSION

This study describes in quantitative terms the changes of arterial inflow, venous outflow and tissue volume in the penis and their time characteristics during pelvic nerve induced erection in the dog. Such combined information about penile circulatory events, not available in the previous literature, may aid in the understanding of the haemodynamics of erection.

For a discussion of this problem reference may first be made to the principal patterns of response demonstrated in Figs. 1 and 2. It appears from those data that pelvic nerve induced erection is accomplished by two main circulatory events. Upon stimulation, there was first a prompt and pronounced dilatation of the penile 'resistance vessels' causing a greatly increased arterial inflow of blood which, however, in the very early (<20 s) phase caused no sign of erection and, hence, bypassed the cavernous bodies, thus increasing venous outflow to the same extent. One consequence of this 'resistance vessel' dilatation must be that arterial microvascular pressure is greatly increased. The second event was the erectile response proper which started with a delay of about 20 s (Fig.

1). Apparently it was caused by a sudden opening of low resistant 'shunt vessels' diverting part of the further increased arterial inflow into the cavernous bodies at established high pressure head from the microvessels, thereby causing rapid filling. During the phase of cavernous filling, arterial inflow greatly exceeded venous outflow (Fig. 2) but it returned to the venous outflow level again in the steady state of full erection. The erection was then well maintained at this flow level of about $110 \text{ ml} \times \text{min}^{-1} \times 100\text{g}^{-1}$, reflecting decreased resistance in both 'resistance vessels' and 'shunt vessels'.

A complete redistribution of blood flow from the extracavernosal vascular system to the cavernous bodies by some constrictor mechanism need not be invoked. If this had occurred it should have been observed as a drastic decrease in venous outflow during the phase of cavernous filling. In fact there was only a minor transient drop in venous outflow during this period (Fig. 2) which most likely is explained merely by passive elastic vascular recoil at the time of shunt opening. Effects of penile vein occlusion described in Results supported the view that the 'shunt vessels' are closed at most times in the flaccid control state.

The 'shunt vessels' to the cavernous bodies, considered to be the penile helicine arteries (Christensen, 1954; Newman & Northup, 1981) are estimated from microsphere studies to have a lumen diameter of about $100 \text{ } \mu\text{m}$ when open (Newman, Northup & Devlin, 1964). The pressure in microvessels of this size can be calculated to rise to 100 mmHg , or more, at maximal resistance vessel dilatation (cf. Fronek & Zweifach, 1975; Grände, Lundvall & Mellander, 1977). Such a pressure per se in the helicine arteries would suffice to produce a cavernous tissue pressure of roughly the same magnitude during full erection, which is compatible with the directly observed penile tissue pressure in this state of about 100 mmHg reported by Henderson & Roepke (1933). Blood flow at high intracavernous pressure is thus evidently flowing through the cavernous bodies to the venous side during full erection, and a venoconstrictor mechanism is not required for cavernous filling as evidenced by Dorr & Brody (1967). An apparent prerequisite for rapid erection, as noticed in copulative behaviour, is that the driving pressure from the penile arterial microcirculation is high at the time of cavernous shunt opening and such an effect seems to be ensured by the described 'preceding' 'resistance vessel' dilatation.

Cessation of nerve stimulation led to quick penile detumescence (Fig. 1) and rapid recovery of tone in the penile 'resistance vessels' as

evidenced by the marked decline in arterial inflow (Fig. 2). Detumescence also seemed to be associated with an early closure of the cavernous 'shunt vessels' as indicated by the findings (Figs. 1 and 2) that the penile volume curve declined very rapidly and that its shape was perfectly smooth without any sign of sudden increase at the time of the large oscillation of arterial inflow noted during detumescence.

It is quite likely that the dynamics of the cavernous filling/emptying during penile erection/detumescence are influenced by an additional factor, viz. the contractile state of the smooth muscles of the cavernous trabeculae (see Sjöstrand & Klinge, 1979). Relaxation of these structures would facilitate blood accumulation in the cavernous bodies during erection and contraction would help to increase the rapidity of detumescence. The fact that the rate of cavernous emptying was faster than the rate of filling (Fig. 1) suggests a significant involvement of the latter mechanism in penile detumescence.

Analysis of the frequency-response characteristics of the penile blood flow response and the erectile response to pelvic nerve stimulation in the dog showed that the threshold frequency for the former was 1-2 Hz and for the latter 2-4 Hz and that maximal effects for both were reached at 16 Hz (Figs. 3 and 5). A maximum erectile response was reported to be obtained at much lower rates of pelvic nerve excitation (2 Hz) in the rabbit (Sjöstrand & Klinge 1979) which indicates a marked species difference. Such a difference might be related to different copulation behaviour in the dog and the rabbit. In the rabbit this act is very shortlasting with sexual arousal and copulation often completed within less than 20 s.

The fact that the penile blood flow response and the erectile response in the dog differed both with regard to time of onset and threshold frequency indicated that the underlying vascular effects were governed by partly different regulatory mechanisms. This hypothesis was strongly corroborated by the comparison of these responses to pelvic nerve stimulation evoked in the absence and presence of atropine which showed that muscarinic blockade caused no significant alteration of the flow response but clearly depressed the erectile response over the entire effective frequency range (Fig. 5). These data, taken together, indicate that the neurotransmitter mechanism for dilatation of the penile 'resistance vessels' is mainly non-cholinergic in nature whereas a cholinergic mechanism seems to contribute to the erectile response proper by an inhibitory influence on the penile 'shunt vessels' and/or the

trabeculae of the cavernous bodies. The existence of muscarinic receptors in penile vascular smooth muscle in the dog was further supported by the observed erectile effects of infused acetylcholine. A cholinergic mechanism in pelvic nerve induced penile erection has been questioned by Klinge & Sjöstrand (1974) on the basis of previous studies by others using supramaximal frequencies (> 20 Hz) of preganglionic pelvic nerve stimulation. It was argued that such high frequency stimulations could cause excess release of acetylcholine activating ganglionic muscarinic receptors and that such an effect could have been blocked when atropine was applied. Although such a possibility cannot be entirely ruled out, that explanation seems to be contradicted by the fact that in the present study the depression of the erectile response by atropine was observed over the entire physiological frequency range of pelvic nerve stimulation (Fig. 5).

The conclusion that the pelvic neural dilatation of the penile 'resistance vessels', i.e. the blood flow response, is non-cholinergic in nature is corroborated by previous studies (Henderson & Roepke 1933; Dorr & Brody 1967; Sjöstrand & Klinge 1979). The present study demonstrated that pelvic nerve stimulation caused a substantial release of VIP from the penis which was correlated in onset and duration to the neural vasodilator response (Fig. 6). I.a. infusion of VIP elicited moderate erection, and a penile vasodilator response which resembled the neural response. These functional data obtained in vivo viewed in the light of previous evidence from morphological and in vitro studies (see Introduction), indicate that VIP might be a neurotransmitter responsible for non-cholinergic pelvic nervous vasodilatation of the penile 'resistance vessels'. Analogous functional evidence for VIP as a neurotransmitter in the pelvic nerves was recently obtained in the cat, both with regard to neural vasodilatation in the penis (Mellander, Andersson & Bloom, 1984) and in an adjacent tissue, the rectum (Andersson, Bloom, Edwards, Järnult & Mellander 1983). The present results provided poor evidence, however, for suggesting substance P as a neurotransmitter involved in pelvic nervous penile vasodilatation.

Although beyond the scope of the present study, it might be emphasized that erection under physiological conditions probably is associated with a complex autonomic activity pattern in the pelvic and hypogastric nerves and in the sympathetic chain fibres. Such aspects were dealt with in a study on rabbits by Sjöstrand & Klinge (1979). One should also be aware of possible species differences in the haemodynamics of erection.

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